

WW domain-containing oxidoreductase's role in myriad cancers: Clinical significance and future implications

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Abstract

The WW domain-containing oxidoreductase (WFOX) gene, encodes a tumor suppressor located on 16q23.1, spanning FRA16D, one of the most active common fragile sites in the human genome, that is altered in numerous types of cancer. WFOX's alteration in these myriad cancers is due to disparate mechanisms including loss of heterozygosity, homozygous deletion and epigenetic changes. *In vitro*, WFOX has been found to be reduced or absent in numerous cancer cell lines and WFOX restoration has been found to inhibit tumor cell growth and invasion. *Wfox* knockout mice developed femoral focal lesions resembling osteosarcomas within one month of their life and aging *Wfox* heterozygous mice have an increased incidence of spontaneous lung and mammary tumors as well as B-cell lymphomas. We herein review WFOX's role that has been unearthed thus far in different types of malignancies, its clinical significance and future implications.

Keywords: WW domain-containing oxidoreductase, tumor suppressor gene, breast cancer, lung cancer, hypermethylation

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Introduction

The WW domain-containing oxidoreductase (WFOX) gene encodes a 46 kDa tumor suppressor that is altered in myriad types of cancer. WFOX is located on 16q23.1, spanning FRA16D, one of the most active common fragile sites in the human genome. The protein which it encodes is comprised of 2 WW domains and a short chain dehydrogenase reductase domain.¹ WFOX was initially discovered as researchers were interested in mapping the 16q region, an area commonly affected by allelic loss in breast cancer. Upon mapping this genomic region, WFOX (also known as *FOR*) was identified and cloned.^{2,3} Chang and colleagues also isolated and characterized WFOX (referred as *WOX1*) as an effector of hyaluronidase that increases cancer cell sensitivity to tumor necrosis factor (TNF) cytotoxicity.⁴ WFOX was recently identified as one of the most significant somatic copy number alterations (SCNA) undergoing deletions in Beroukhi *et al.*'s high resolution analysis of a plethora of cancer specimens.⁵ Some reports, however, documented increased levels of WFOX in breast, gastric and prostate carcinomas suggesting a complex regulation of WFOX in cancer.^{6,7} Nevertheless, the vast majority of observations support loss of WFOX expression in cancer

and indicate a tumor suppressive role of its protein product as shall be discussed here.

WFOX's alteration in these myriad cancers is due to disparate mechanisms including loss of heterozygosity (LOH), homozygous deletion, hypermethylation of the regulatory region and, in one study due to ubiquitination. These WFOX alterations have been associated with poorer prognoses in different types of malignancies (below). These facts have exciting therapeutic implications.

In vitro, WFOX has been found to be reduced or absent in numerous cancer cell lines and WFOX restoration has been found to inhibit invasion and to suppress tumorigenicity in xenograft mouse models.^{8–12} Mice carrying a targeted deletion of *Wfox* developed spontaneous lung papillary carcinomas, in the case of *Wfox*-heterozygous mice and osteosarcomas in the case of *Wfox* null mice.¹³ More recently, it was shown that 50% of female *Wfox*-heterozygous (C3H) (WFOX (C3H)+/–) mice formed mammary tumors.¹⁴ *Wfox* hypomorphic mice displayed shorter lifespans with the males enduring testicular atrophy and reduced fertility and the females having an increased incidence of spontaneous B cell lymphomas.¹⁵ Altogether, these observations indicated that WFOX acts as a bone fide tumor suppressor (reviewed in Refs. 16–18).

Causes of WWOX alteration

WWOX's alteration is due to disparate phenomena (Table 1). Genomic instability and mutation was dubbed one of the two enabling characteristics of Hanahan and Weinberg's hallmarks of cancer.¹⁹ Although point mutations are not frequent, some mutations have been reported in WWOX's coding region.²⁰ LOH and homozygous deletions of WWOX have both been reported in several cancer types. Hypermethylation, an epigenetic mechanism, has also been identified as a means of WWOX alteration and attenuation. Ubiquitination and subsequent degradation has also been described as a cause of WWOX attenuation. In head and neck squamous cell carcinoma, it was reported that *oncomiR-134* downregulates WWOX suggesting that microRNAs can also regulate WWOX expression.²¹

Loss of heterozygosity

LOH at the WWOX locus has been described in numerous malignancies (Table 1), including breast, lung and gastric cancer.^{22,23} LOH was demarcated in the 16q23 region in 67%, 53% and 52% of breast, prostate and hepatocellular carcinomas, respectively.²⁴ LOH at the WWOX locus was found in 45.6% of gastric cardia adenocarcinoma samples.²⁵

Homozygous deletion

Finnis *et al.* detected homozygous deletions within WWOX in three colon cancer derived cell lines (Co115, KM12C and KM12SM).²⁶ Homozygous deletion of WWOX exons from an ovarian cancer cell line, two small cell lung cancer cell lines and a pancreatic cancer cell line were reported as well.²⁴

Hypermethylation

Epigenetics has been underscored as an influential factor in tumorigenesis. Hypermethylation in a tumor suppressor gene's promoter region can silence that gene's expression and consequently give the cells a growth advantage.²⁷ Iliopoulos *et al.* demonstrated WWOX promoter region hypermethylation as a means of WWOX inactivation in breast, lung and bladder neoplasias. A correlation between WWOX CpG methylation and reduced WWOX protein expression in all three types of cancers was described.²⁸ In a later study, Iliopoulos performed methylation specific PCR of the WWOX promoter region on three breast cancer cell lines, MCF7 which expresses WWOX strongly, and MDA-MB-231 and HCC1937 and found both promoter

regions unmethylated in the MCF7 cells and methylated in the MDA-MB-231 cells.⁹ Wang *et al.* found similar results regarding hypermethylation of WWOX promoter CpG islands and decreased WWOX expression in breast carcinoma samples and two cell lines.²⁹

Ubiquitination

Ubiquitination, an enzymatic cascade whereby proteins are labeled with a ubiquitin tag and thus designated for degradation via the proteasomal system, is another means by which WWOX could be inactivated and thereby subsequently promote tumorigenesis.³⁰ Mahajan *et al.* demonstrated that Ack1, an intracellular tyrosine kinase induces WWOX polyubiquitination and subsequent degradation in prostate cancer cells. In that same study human specimens of androgen independent prostate cancers (AICaP) and benign prostate tissue were obtained and immunoprecipitation and immunoblotting revealed that AICaP had increased levels of phosphorylated Ack1 and reduced levels of WWOX compared to the benign prostate samples, suggesting WWOX degradation due to tagging for ubiquitination by Ack1.³¹ So far no ubiquitin E3 ligase was associated with WWOX under normal or disease states.

WWOX alteration in cancer

Breast cancer

Breast cancer is the most common cancer among women worldwide and its incidence is rising.³² There have been numerous studies done underscoring the role of WWOX alteration in breast cancer. A locus within WWOX has even been identified as a genetic breast cancer risk variant.³³ WWOX has been shown to be of significant importance in mammary gland physiology as WWOX ablation in mice is associated with increased fibronectin levels and impaired ductal growth.³⁴ In *Wwox* (C3H)+/- mice 50% of female mice formed tumors while only 7% of female wild type mice did. Furthermore, it has been reported that among those *Wwox* (C3H)+/- mammary tumors, WWOX expression was often lost suggesting LOH, a hallmark of tumor suppressors.¹⁴ Guler *et al.* were the first to notice the correlation between reduced WWOX expression in human breast cancer samples and breast carcinogenesis. Reduced WWOX expression was reported in 63.2% of breast cancer specimens.³⁵ In a study analyzing WWOX mRNA expression in 9 breast cancer cell lines and 20 human breast tumor samples, reduced WWOX expression

Table 1 WWOX alterations in disparate cancers

Alteration	Cancer type and references
LOH	Breast ²⁴ , lung ²² , gastric ^{23,25} , prostate ^{24,54} , hepatocellular ²⁴ , pancreatic ⁷³
Homozygous deletion	Lung ²⁴ , ovarian ²⁴ , pancreatic ²⁴ , colon ²⁶
Hypermethylation	Breast ^{9,28,29} , lung ^{28,57,62} , prostate ¹⁰ , bladder ²⁸ , pancreatic ^{73,76} , gastric ^{23,78}
Ubiquitination and degradation	Prostate ³¹

LOH: loss of heterozygosity.

in 3 cell lines was found as were *WWOX* mRNA variants in a number of the tumors.³⁶ In addition to the initial observation by Bednarek *et al.*,^{2,37} these data paved the road for a possible tumor suppressor function of *WWOX* in breast cancer.

There have been several studies demonstrating *WWOX* restoration *in vitro* and *in vivo* and subsequent inhibition of breast cancer cell growth. Bednarek *et al.* noted *WWOX*'s ability to inhibit tumorigenicity both *in vitro* and *in vivo* using MDA-MB-435 breast cancer cell lines.³⁷ Huebner's lab elegantly displayed this phenomenon as well and found that exogenous *WWOX* restoration inhibited breast cancer cell growth *in vitro* and *in vivo* and endogenous *WWOX* restoration via *de novo* methyltransferase inhibitor treatment inhibits breast cancer cell growth and shrinks xenograft tumor growth.⁹ A mechanism for *WWOX*'s tumor suppressor role was proposed by Aqeilan *et al.* who demonstrated *WWOX*'s relationship with Ap2gamma transcription factor, an oncogene in breast cancer and suggested *WWOX* precludes its activity by sequestering it in the cytoplasm.³⁸

WWOX expression has been significantly associated with a number of clinicopathologic factors in breast cancer. *WWOX* reduction is significantly correlated with that of fragile histidine triad (FHIT), encompassing the most active common fragile site FRA3B,³⁹ in breast cancer specimens.³⁵ Reduced FHIT and *WWOX* were found to be associated with ductal carcinoma *in situ* (DCIS).⁴⁰ Subsequently, it was established that expression of *WWOX* and FHIT is significantly reduced in triple negative breast cancer specimens, a more aggressive breast cancer type.⁴¹ An association between *WWOX* expression, intramembranous ErbB4 expression (in contradistinction to intranuclear expression) and increased disease free survival has been reported.⁴² Conversely, loss of *WWOX* expression was associated with increased metastatic axillary lymph nodes and decreased disease free survival.⁴² In agreement to these observations, Guler *et al.* found significantly reduced *WWOX* and ErbB4 in metastases compared to their matched primary breast tumor.⁴¹ Nunez *et al.* found a correlation between estrogen receptor status and *WWOX* expression. A greater number of estrogen receptor (ER)-breast cancer samples, a subtype associated with a poorer prognosis,⁴³ had absent or weakly staining *WWOX* expression.⁴⁴ Pluciennik *et al.* delineated the same phenomenon and found it to be true regarding progesterone receptor status as well. Conversely, higher *WWOX* expression was found to be associated with greater disease free survival.⁴⁵ Wang *et al.* found *WWOX* to be associated with a basal like phenotype of breast cancer as well as decreased disease free survival.⁴⁶

A significant association between *WWOX* and tamoxifen resistance has been described. This study suggested that *WWOX* and Ap2gamma may be more telling biomarkers regarding tamoxifen efficiency than progesterone receptor and Her2 status.⁴⁷ Gothlin *et al.* found an association between increased *WWOX* expression and decreased risk of recurrence in patients treated with tamoxifen.⁴⁸

Decreased *WWOX* expression in breast cancer has been significantly associated with a number of clinicopathologic parameters such as more aggressive tumor phenotypes,

increased metastases and decreased disease free survival. Therefore, *WWOX* might be a promising biomarker to gauge chemotherapy affectivity. This has exciting implications regarding *WWOX*'s clinical role in the future.

Prostate cancer

Prostate cancer accounts for 6% of cancer deaths among males worldwide.⁴⁹ Prostate cancer derived cells were found to have decreased *WWOX* mRNA and protein expression and 84% of prostate cancer samples were found to have decreased *WWOX* expression via immunohistochemical staining. *WWOX* overexpression *in vitro* induced apoptosis via a caspase-dependent mechanism and halted cell growth. Ectopic *WWOX* expression was found to suppress tumorigenicity in xenografts.¹⁰ One mechanism by which *WWOX* exerts its tumor suppressor role in prostate cancer is by binding to Ap2gamma in the cytoplasm, precluding it from binding with ErbB2's (an effector of prostate cancer cell growth) promoter region in the nucleus.⁵⁰ In prostate cancer cells, *WWOX* itself might be activated by complement C1q as suggested by Hong *et al.*'s study.⁵¹

WWOX was identified as an area of genomic loss via array comparative genetic hybridization of 16 prostate tumor samples.⁵² A single-nucleotide polymorphism (SNP)-based genome wide linkage scan of 161 families with prostate cancer detected a strong signal on 16q23, which could very well be *WWOX*.⁵³ *WWOX* promoter hypermethylation was noted in prostate cancer derived cells. Increased *WWOX* expression in prostate cancer derived cells was delineated after treatment with AZA, a DNA methyltransferase inhibitor and trichostatin A, a histone deacetylase inhibitor. Intratumoral AZA treatment suppressed tumor growth in nude mice.¹⁰ As mentioned above, another means of *WWOX* inactivation in prostate cancer is its phosphorylation by Ack1, tagging it for polyubiquitination and subsequent degradation.³¹ In a CNA study of prostate tumors, 17.7% were found to have LOH of *WWOX* which was significantly associated with a more advanced disease staging.⁵⁴ Further associations between reduced or lost *WWOX* and other clinicopathologic parameters including disease free survival must be explored.

Lung cancer

Lung cancer is the most common cause of cancer death among males worldwide.⁴⁹ A number of studies have delineated *WWOX* alteration in lung cancer. Yendamuri and colleagues initially analyzed 8 non-small cell lung cancer (NSCLC) cell lines and 27 normal lung and lung tumor tissues for *WWOX* alterations. They found missing *WWOX* transcripts in 25.9% of primary tumors and five out of eight cell lines. Thirty-seven percent of the primary tumors displayed LOH at the *WWOX* locus.²² *WWOX* expression was absent or reduced in 84.9% of NSCLCs in a study of 170 samples and present in 80.5% of adjacent normal lung tissue.⁵⁵ A study of the tissue of 44 Chinese patients who had undergone resections for NSCLC found 63.6% of samples to have loss of *WWOX* exons 6-8 transcript.⁵⁶ In a different study of 50 lung cancer patients, 2 germline mutations and 2 polymorphic mutations were

isolated in WWOX upon DNA sequencing.⁵⁷ WWOX restoration in WWOX protein negative lung cancer cell lines *in vitro* and in nude mice xenografts *in vivo* induced apoptosis and a striking suppression of tumorigenicity, respectively.^{8,11,58}

The degree of WWOX expression was found to correlate with histopathologic features of NSCLC including histiotype and increased tumor aggressiveness. Weak WWOX staining was associated with high cell proliferation and poor differentiation.⁵⁵ WWOX deletion was significantly higher in the tumors of those patients who smoked.⁵⁶ WWOX preservation in resected NSCLC tumors of 85 patients was associated with better outcomes.¹¹ Certain SNPs within WWOX were identified as being significantly associated with increased cancer risk.⁵⁹ A common WWOX copy number variation isolated among the Chinese was found to significantly increase the carrier's likelihood of developing lung cancer.⁶⁰

WWOX promoter hypermethylation was identified in 76% of 50 lung cancer patients.⁵⁷ Epigenetic modulation of nude mice with H1299 lung cancer tumors (which lack FHIT and WWOX) via AZA (a demethylating agent) injection restored WWOX (among other tumor suppressor genes) expression and decreased tumorigenicity, as well as increased the apoptosis fraction, increased caspase 3 activation and decreased mitosis in those tumors that responded.⁶¹ Another study identified the downregulation of the microRNA 29 family as causing epigenetic WWOX attenuation by allowing DNMTA upregulation which hypermethylates WWOX's promoter region and silences WWOX. Upon forced miR-29 expression in NSCLC, WWOX and other tumors suppressor genes affected by hypermethylation were found to be reexpressed and tumorigenicity was found to be inhibited *in vitro* and *in vivo*.⁶² Another means of epigenetic WWOX modulation in lung cancer was proposed by Kimura *et al.* who found that Bmi1, an epigenetic regulator protein which is highly expressed in SCLC, targets WWOX thereby attenuating it. Bmi1 deletion caused increased apoptosis presumably via restored WWOX functioning.⁶³

Osteosarcoma

Osteosarcoma comprises 35% of all primary bone tumors. It is the most common primary malignant bone tumor in children and adolescents. While long-term survival for nonmetastatic osteosarcoma is 60–70%, it is only around 30% for patients with metastases.⁶⁴ WWOX has been found to play a role in osteosarcoma. As referred to above *Wwox* knockout mice develop focal lesions resembling osteosarcomas within their life of one month.^{13,65,66} WWOX was found to be deleted in 30% of human osteosarcoma samples⁶⁷ and its protein is lost or reduced in 58% of human osteosarcoma samples.⁶⁵ Decreased WWOX mRNA was detected by RT-PCR and associated with malignancy in a number of osteosarcoma samples.⁶⁸ Furthermore, WWOX levels were increased in tumors resected post chemotherapy in comparison to primary tumors.⁶⁵ WWOX levels were decreased in lung metastases in comparison to levels in the primary tumor. WWOX was reduced in human

osteosarcoma cell lines as well. WWOX restoration inhibited proliferation and modified invasion capability *in vitro* and suppressed tumorigenesis *in vivo*. RUNX2, a transcription factor responsible for bone development and regulation and a WWOX binding partner,⁶⁹ was found to be elevated in the femurs of *Wwox* knockout mice. RUNX2, which displays elevated levels in osteosarcoma,⁷⁰ expression was found to be elevated in osteosarcoma cell lines with reduced WWOX expression. RUNX2 expression was positive in most clinical osteosarcoma samples and negative in most post chemotherapy samples. Additionally, WWOX restoration in osteosarcoma cell lines was associated with RUNX2 downregulation. These findings support the notion that WWOX's binding with RUNX2 inhibits RUNX2's transactivation function.⁶⁵ Del Mare *et al.* further explicates the WWOX-RUNX2 functional cross talk and its implications for understanding the pathogenesis of osteosarcoma.⁷¹

Pancreatic cancer

Pancreatic cancer, one of the most lethal cancers, has been deemed the fourth leading cause of cancer death. Pancreatic resection is the only curative option, yet most patients present too late to be candidates for that procedure. Those patients who do undergo pancreaticoduodenectomies only have a dismal five year survival rate of anywhere from 10% to 25%.⁷² Kuroki *et al.* studied WWOX expression and promoter methylation in paired pancreatic cancer samples as well as in pancreatic cancer cell lines. The study found LOH in the WWOX locus in 27% of primary tumors and WWOX promoter hypermethylation in 13% of primary tumor samples and in 22% of pancreatic cancer cell lines. Significant reduction in WWOX mRNA expression and WWOX protein levels was found in all of the cell lines. Forty percent of primary tumors were found to have a significant reduction in WWOX expression as well. Pancreatic cell colony formation was inhibited upon WWOX transfection via apoptosis.⁷³ WWOX restoration inhibited further cell growth *in vitro* and *in vivo*. Not only was cancerous tissue found to have reduced WWOX, but precancerous pancreatic cancer tissue samples were found to have decreasing levels of WWOX expression in accordance with their pancreatic intraepithelial neoplasia grade and with eventual less favorable patient outcomes. Normal pancreatic tissue from the surrounding tissue of the samples was found to have a robust WWOX expression.⁷⁴ Bloomston *et al.* found reduced WWOX and FHIT expression in pancreatic, gall bladder and ampullary cancers and robust WWOX expression in physiologic tissue as well. They did not, however, find any correlation between the gradation of WWOX expression and tumor progression or patient survival.⁷⁵ Nakayama *et al.* studied 41 intrapapillary mucinous neoplasms of the pancreas to further delineate WWOX's role in pancreatic tumorigenesis and found WWOX loss or reduction in 15% of intrapapillary mucinous adenomas (IPMAs) and 81% of intrapapillary mucinous carcinomas (IPMCs). Furthermore, hypermethylation of the WWOX promoter region was found in 33% of WWOX negative IPMAs and 53% of WWOX negative IPMCs.⁷⁶

Gastric adenocarcinoma

Gastric cancer is responsible for 10% of cancer deaths globally.⁴⁹ Aqeilan *et al.* were the first to note WWOX alteration in gastric adenocarcinoma. LOH in 31% of primary tumors and loss of WWOX protein expression in 65% of primary tumors and 33% of gastric adenocarcinoma cell lines was described.²³ Maeda *et al.* also found loss of WWOX expression in gastric cancer specimens and cell lines. Hypermethylation at the WWOX regulatory site was appreciated in two of the cell lines.⁷⁷ Furthermore, WWOX promoter and exon 1 methylation was noted in gastric cardia adenocarcinoma specimens.²⁵ Additionally, WWOX hypermethylation has been correlated with *Helicobacter pylori* infection. Yan *et al.* suggested based on this and other findings that *H. pylori* induces WWOX hypermethylation and subsequent gastric carcinogenesis.⁷⁸ Takeuchi *et al.* suggested another means by which WWOX function might be attenuated in gastric signet cell carcinoma. They isolated a gene, transmembrane protein 207 (TMEM 207), which they found to be expressed in a gastric signet ring cell carcinoma cell line as well as in a number of tissue samples. They found it to bind with WWOX upon coimmunoprecipitation. They also noted WWOX inhibition of invasion in a different signet ring cell carcinoma cell line to be attenuated upon TMEM 207 overexpression.⁷⁹ A correlation between WWOX and FHIT expression has been discovered in gastric adenocarcinoma as well.²³ Upon intragastric administration of *N*-nitrosomethylbenzylamine to *Wwox*-heterozygous (WWOX+/-) and *Wwox*-wild type (WWOX+/+) mice and subsequent analysis, 96% of WWOX+/- mice had developed forestomach tumors whereas only 29% of WWOX+/+ mice did. Furthermore, 29% of WWOX+/- mice developed invasive squamous cell carcinoma of the forestomach whereas none of the WWOX+/+ mice did. This study suggests that loss of one WWOX allele enhances tumorigenicity.⁸⁰ WWOX restoration into a gastric adenocarcinoma cell line effectively suppressed cell growth and increased the number of cells in the subG1 stage of the cell cycle. The degree of WWOX expression was found to be significantly correlated with clinical staging, depth of invasion, lymph node status, metastases and venous infiltration.⁷⁷ These data indicate that WWOX may play a role in gastric carcinogenesis and furthermore may be a valuable biomarker in terms of gauging clinical features of tumor aggressiveness.

Ovarian cancer

Ovarian cancer is the seventh most common cancer in women globally.⁴⁹ In a comparison between normal and malignant ovarian tissue, normal tissue was found to have strong WWOX expression whereas ovarian carcinoma tissue was found to have reduced expression.^{81,82} A homozygous deletion within WWOX was detected in an ovarian cancer derived cell line with the corresponding aberrant transcript and lack of full WWOX product.²⁴ WWOX restoration or overexpression, depending on the cell line, inhibited tumorigenicity *in vivo*. Interestingly, WWOX restoration or overexpression *in vitro*, caused decreased attachment and migration onto fibronectin, an extracellular membrane component integral to peritoneal metastasis.

WWOX knockdown in ovarian cancer cell lines with endogenous WWOX expression caused increased fibronectin adhesion. Based on these *in vitro* findings, Gourley *et al.* proposed loss of WWOX as an important factor in peritoneal metastasis in ovarian cancer.¹² Xiong *et al.* found WWOX overexpression *in vitro* to induce apoptosis and inhibit further ovarian cancer cell growth.⁸³

A significant association between reduced WWOX expression and overall survival as well as clinical staging (FIGO stage IV), estrogen and progesterone receptor negative status and lymph node metastasis has been reported.^{81,84} Eight previously identified SNPs within WWOX were genotyped in 554 ovarian cancer samples and analyzed for association with clinicopathologic factors such as tumor grade and survival. Three SNPs were identified as being significantly associated with one or more clinicopathologic features including tumor grade, histology and progression free survival. These associations, however, were not confirmed in a different ovarian cancer population.⁸⁵ In a study evaluating sodium valproate as a treatment for ovarian cancer in ovarian cancer cell lines and xenografts, WWOX was used as a marker to determine treatment efficacy, as WWOX protein expression increased post treatment in ovarian cancer cell lines and WWOX was upregulated in xenografts post treatment as well.⁸⁶

Squamous cell carcinoma

Cancers of the skin, including melanoma and basal and squamous cell skin cancers, are by far the most common malignancies in humans.⁸⁷ The incidence of these types of skin cancer has increased greatly over the past decade. However, the etiology is largely unknown. Unlike basal cell carcinoma, cutaneous squamous cell carcinoma (SCC) is malignant and tends to metastasize. Lai *et al.* noted that WWOX expression is induced during normal keratinocyte differentiation suggesting a physiological role in skin biology.⁸⁸ However, in the vast majority of SCC cases, WWOX protein levels are reduced, while WWOX mRNA was not reduced at all and thus suggested that cancerous cells inhibit WWOX mRNA's translational capacity.⁸⁸ Interestingly, exposure of SCC cells to UVB or methotrexate enhanced WWOX expression, sensitized the cells to undergo apoptosis and inhibited autophagy.⁸⁹ Conversely, WWOX depletion in these cells rendered them more resistant to apoptosis, a hallmark of cancer cells. *In vivo*, acute exposure of hairless mice to UVB was associated with upregulation of WWOX in epidermal cells in 24 h. In accordance with WWOX loss in human SCC, chronic UVB-treated mice developed cutaneous SCCs in three months, with significant reduction of WWOX protein levels and no effect on WWOX mRNA.⁸⁸ This notion that WWOX levels are increased upon UVB radiation may suggest that WWOX might be activated upon DNA damage and/or play a role in DNA damage response.

Clinical significance

WWOX is portended to be an important biomarker in numerous cancers. WWOX has already been denoted as a significant factor in determining the clinical outcome of patients with multiple myeloma.⁹⁰ As noted above the

Table 2 WWOX alteration in different cancers and its clinical significance

Cancer type	Alterations	Correlations/Conclusions	References
Breast	Reduced WWOX expression in 63.2%	Correlated with less favorable ER status	Guler <i>et al.</i> ³⁵
	Reduced FHIT and WWOX expression in 61% of adjoining invasive tumors	Reduced FHIT and WWOX positively associated in DCIS	Guler <i>et al.</i> ⁴⁰
	Wwox expression absent in 36%	Associated with unfavorable outcome	Aqelian <i>et al.</i> ⁴²
	WWOX and ErbB4 expression reduced in metastatic vs. matched primary tissue	Suggests that WWOX loss contributes to LN metastasis	Guler <i>et al.</i> ⁴¹
	WWOX expression absent in 46% of ER– breast carcinomas, absent or weakly staining in 73% ER–	WWOX is correlated with ER status and thus poorer prognosis	Nunez <i>et al.</i> ⁴⁴
Prostate	Wwox expression increased in a number of breast cancer samples	Increased disease free survival	Pluciennik <i>et al.</i> ⁴⁵
	WWOX absent in 29% of breast carcinomas	Associated with decreased disease free survival	Wang <i>et al.</i> ⁴⁶
	Mean WWOX levels significantly lower in basal-like breast cancers		
	WWOX significantly reduced in tamoxifen resistant breast cancer samples	Suggests role for WWOX as biomarker to predict tamoxifen resistance	Guler <i>et al.</i> ⁴⁷
	17.7% WWOX LOH in prostate tumors		
Lung	25.9% WWOX transcripts absent, 37% WWOX LOH in NSCLCs	Significantly associated with more advanced disease stage	Cheng <i>et al.</i> ⁵⁴
	WWOX expression was absent or reduced in 84.9% of NSCLCs	Suggests WWOX reduction's important role in NSCLC	Yendamuri <i>et al.</i> ²²
	WWOX expression preserved in a number of NSCLCs	WWOX reduction associated with more aggressive histologic tumor type	Donati <i>et al.</i> ⁵⁵
	Loss of WWOX exons 6–8 transcript in 63.6% NSCLCs	Associated with improved outcome	Becker <i>et al.</i> ¹¹
	WWOX promoter hypermethylation in 76% lung cancers	Suggests role for WWOX exons 6–8 in NSCLC pathogenesis	Zhou <i>et al.</i> ⁵⁶
Osteosarcoma	WWOX lost or reduced in 58% of osteosarcomas	Further evidence for WWOX's role in lung tumorigenesis	Baykara <i>et al.</i> ⁵⁷
	WWOX levels increased in post chemotherapy OS tumors	Further promotes WWOX's role in OS pathogenesis	Kurek <i>et al.</i> ⁶⁵
	WWOX deletion is 30% of osteosarcomas	Loss of WWOX expression is an early event in the pathogenesis of osteosarcoma	Yang <i>et al.</i> ⁶⁷
	LOH within WWOX locus in 27% of pancreatic tumors and WWOX promoter hypermethylation in 13%	Establishes WWOX's role in pancreatic cancer carcinogenesis	Kuroki <i>et al.</i> ⁷³
	WWOX expression reduced in both pancreatic cancers and precancerous pancreatic lesions	Degree of reduction correlated with grade and poorer outcomes	Nakayama <i>et al.</i> ⁷⁴
Pancreatic	WWOX loss or reduction in 15% of intrapapillary mucinous adenomas (IPMAs) and 81% of intrapapillary mucinous carcinomas (IPMCs)	Further establishes WWOX's role in pancreatic cancer	Nakayama <i>et al.</i> ⁷⁶
	Hypermethylation of the WWOX promoter region in 33% of WWOX negative IPMAs and 53% of WWOX negative IPMCs		
	LOH in 31% of primary gastric tumors and loss of WWOX protein expression in 65% of primary tumors	Established a role for WWOX in gastric cancer	Aqelian <i>et al.</i> ^{23,38}
	Hypermethylation at WWOX regulatory site and absent WWOX expression in 33% of gastric carcinomas	Correlated with clinical staging, depth of invasion, lymph node status, metastases and venous infiltration	Maeda <i>et al.</i> ⁷⁷
	Absent or barely detectable WWOX expression in 30% of ovarian carcinomas	Significantly associated with stage IV, negative PR status and shorter overall survival	Nunez <i>et al.</i> ⁸¹
Ovarian	Loss of WWOX expression in 28.6% of ovarian carcinomas	Correlated with advanced FIGO stages, lymph node metastasis, negative ER and negative PR status	Lan <i>et al.</i> ⁸⁴
Colorectal	High level of WWOX expression found in a number of colorectal cancer samples	Correlated with better disease free survival	Zelazowski <i>et al.</i> ⁹⁸

(continued)

Table 2 Continued

Cancer type	Alterations	Correlations/Conclusions	References
Hepatocellular Wilms' tumor	23/51 tumors had decreased WWOX mRNA Varying WWOX expression levels in Wilms' tumor samples	Suggests a role for WWOX in HCC pathogenesis Increased WWOX expression associated with apoptosis, and signal transduction via ErbB4 and EGFR	Aderca <i>et al.</i> ⁹⁴ Pluciennik <i>et al.</i> ⁹⁷
Adenoid cystic	WWOX and FHIT significantly reduced in a number of ACCs	Suggests loss of WWOX and FHIT may play a role in the pathogenesis of basaloid differentiation in salivary gland cancers	Dincer <i>et al.</i> ⁹⁹
Squamous cell	WWOX expression significantly reduced in SCCs without WWOX mRNA downregulation	Suggests that cancerous cells inhibit WWOX mRNA's translational capabilities	Lai <i>et al.</i> ⁸⁸
Glioblastoma multiforme	WWOX LOH and promoter methylation in GBM samples	WWOX correlated with antiapoptotic marker Bcl2 and marker of cell proliferation Ki67 in GBM, as well as ErbB4 signal transduction	Kosla <i>et al.</i> ¹⁰⁰
Hematopoietic	Absent or abnormal WWOX transcripts detected in 51% of primary hematopoietic neoplasias Significantly reduced WWOX mRNA and expression in a number of leukemia patients	Suggests a role for WWOX loss in hematopoietic neoplasia pathogenesis Further supports WWOX's role as a tumor suppressor gene in leukemia	Ishii <i>et al.</i> ¹⁰² Cui <i>et al.</i> ¹⁰³

LOH: loss of heterozygosity; NSCLC: non-small cell lung carcinoma; HCC: hepatocellular carcinoma; ACC: adenoid cystic carcinoma; SCC: squamous cell carcinoma; GBM: glioblastoma multiforme.

degree of WWOX expression has been shown to be significantly associated with numerous clinicopathological features in a number of disparate malignancies (Table 2). WWOX has been suggested as a possible marker of chemotherapy effectiveness.^{47,48,65} Increasing studies are being done to understand the epigenome and genome of cancer with an eye towards targeting particular genes and creating personalized therapy.⁹¹ A recent study identified shared genomic features between three disparate types of tumors.⁹² WWOX alteration is common in a plethora of cancers as described and WWOX restoration via epigenetic modulation or other means may very well be a powerful tool in contributing to the cure of these many cancers.

Concluding remarks

WWOX research is burgeoning and WWOX's activity in disparate types of neoplasias is ever being discovered. WWOX was found to have a significant role in hepatocellular carcinoma as well, activating the Wnt/beta-catenin pathway when it is downregulated and inducing apoptosis when it is overexpressed.^{93–96} Pluciennik *et al.* found WWOX to be altered in Wilms' tumor.⁹⁷ Zelazowski *et al.* found a significant correlation between increased WWOX expression in colorectal cancer samples and disease free survival.⁹⁸ Dincer *et al.* demonstrated reduced WWOX and FHIT expression in adenoid cystic carcinoma, a basaloid salivary gland neoplasm.⁹⁹ WWOX has been found to play a role in glioblastoma multiforme (GBM) as well. Kosla *et al.* found WWOX LOH and WWOX promoter methylation in GBM. They also found WWOX to be correlated with antiapoptotic marker Bcl2 and marker of cell proliferation Ki67 in GBM.¹⁰⁰ Chiang *et al.* overexpressed WWOX in GBM cell lines with a mutant p53 and reported that WWOX overexpression induces apoptosis.¹⁰¹ Absent or abnormal WWOX transcripts were detected in 51% of primary hematopoietic neoplasias.¹⁰² Cui *et al.* recently further elucidated the role of WWOX in leukemia.¹⁰³ We have had promising preliminary results demonstrating WWOX's role in Ewing's sarcoma as well (unpublished data). WWOX's role in these cancers must be further explored. The fact that WWOX lies in a common fragile site could render it more sensitive to genomic alteration and deletion. One allele could be lost due to genomic alteration (i.e. as a result of replication stress) while the other is selected against and lost during transformation likely due to silencing by hypermethylation or due to LOH. Further characterization of WWOX function in pre-malignant stages is of great interest and would shed light on its role(s) in early stages of transformation. Though we are cognizant of the argument that WWOX loss is secondary due to its localization within a fragile site and not a primary event in cancer pathogenesis, we are convinced that the fact that WWOX restoration in WWOX-negative cells suppresses tumorigenicity *in vitro* and *in vivo* and that *Wwox* knockout mice are more prone to form tumors suggests otherwise. Further research will further our understanding of WWOX biology and its role in cancer pathogenesis. The delineation of WWOX's role(s) in pre-malignancies, where WWOX levels have been shown to be elevated in some tissues, is of great interest and

requires further investigation. WWOX's use as a biomarker to gauge tumor aggressiveness, clinical outcomes and chemotherapy effectiveness could be around the corner. Future translational work involving WWOX restoration in clinical trials in these disparate cancers is an exciting prospect we would like to see in the not too distant future.

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DECLARATION OF INTEREST

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