Alteration of WWOX in human cancer, a clinical view

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Abstract

WWOX gene is located in FRA16D, the highly affected chromosomal fragile site. Its tumor suppressor activity has been proposed on a basis of numerous genomic alterations reported in chromosome 16q23.3–24.1 locus. *WWOX* is affected in many cancers, showing as high as 80% loss of heterozygosity in breast tumors. Unlike most tumor suppressors impairing of both alleles of *WWOX* is very rare. Despite cellular and animal models information on a *WWOX* role in cancer tissue is limited and sometimes confusing. This review summarizes information on *WWOX* in human tumors.

Keywords: *WWOX*, tumor suppressor gene, cancer

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Introduction

Carcinogenesis is a complex, multistep process associated with genetic changes in the human genome. The alterations, such as point mutations, chromosomal deletions, and gene rearrangements, disturb the expression of multiple genes and lead to defects in cell differentiation and division, resulting in cancerous transformation. This is particularly hazardous to the cell when damage occurs to oncogenes, genes that stimulate cell growth and proliferation, or tumor suppressor genes, which inhibit it. Several known oncogenes and tumor suppressor genes exist, such as *TP53* (the genome guardian),¹ *TP16*² and *RB1* (cell cycle gate keepers), and the Ras and Myc families (signal transduction pathways)³ whose aberrations are involved in carcinogenesis and disease progression. Some of these genes, such as *BRCA1* and *BRCA2*,⁴ *RET*,⁵ *ABL*,⁶ and *HER7* are tissue specific. Determining the gene expression profiles of cancer cells and analyzing the relevant protein-protein interaction networks is an important step in understanding the cancer-specific changes which take place in genome structure and functional networks. These are essential for proper diagnosis and the design of effective treatment plans. Therefore, the identification of new genetic/protein factors and gaining an understanding of the relationships which exist in cancer initiation, promotion, and progression remains the goal of many researchers and laboratories.

The *WWOX* protein, also known as WOX1 or FOR (a WW domain-containing oxidoreductase) is a tumor suppressor that has been arousing much research interest. The *WWOX* gene is localized on the long arm of chromosome 16, locus 23.3–24.1 in humans.⁸ It spans the one of the most frequently expressed common human chromosomal fragile

site, FRA16D, which is involved in numerous cancers, such as breast, ovarian, prostate, lung, esophageal, gastric, pancreatic, and hepatic cancer.⁹ As the FRA16D site is highly susceptible to DNA damage, a high rate of translocations, deletions, increased sister chromatid exchanges, and other alterations are observed within it.¹⁰ The *WWOX* gene encompasses approximately 1.1 Mbp. Like any typical gene located in a common fragile site, it possesses very large introns. In normal tissue, the *WWOX* product contains nine exons encoding an open reading frame of 1245 bp.⁸ However, as many tumors use an alternative form of splicing which is extensively disorganized, they often express aberrant isoforms of the *WWOX* gene not found in corresponding normal tissue. The alternative splicing variants are produced by deletions of exons 5–8 and 6–8 in *WWOX* mRNA. On the other hand, the high number of genomic alterations of FRA16D occurring in cancer cells raise doubts whether *WWOX* really is a tumor suppressor gene or whether it is simply a passenger of genetic common fragile site instability, as proposed by Watanabe *et al.*¹¹ who dispute its tumor suppressor function. Watanabe *et al.*¹¹ report that in 48 examples of 49 different cancer cell lines, full-form transcripts and proteins of *WWOX* are observed, which contrast with most genes showing tumor suppressor functions. However, growing evidence from functional studies indicates that *WWOX* plays a role in carcinogenesis and cancer progression. The full-length *WWOX* protein has 414 amino acids, corresponding to a 46 kDa protein. It contains two functional AA-pattern WW domains on the N-terminus and a short chain dehydrogenase domain (SDR).⁸ The WW domains were localized between 18 and 47 as well as 59 and 88 amino acids, respectively. The first WW domain presents

the typical structure of the WW domain: 26 amino acids with two highly conserved tryptophan residues and one proline. In the second WW domain, one tryptophan residue has been replaced by a tyrosine, which is less common in WW domains.⁸ The first WW domain is a main protein-protein interacting domain of WWOX and binds proteins sharing a PPXY motif (P—proline, Y—tyrosine, X—any amino acid).^{12–14} Several protein partners of WWOX have been found so far: P73,¹⁵ AP-2gamma,¹⁶ DVL-2,¹⁷ RUNX2,¹⁸ ERBB4.¹⁹ The SDR domain is responsible for the dehydrogenase activity of the WWOX protein in the presence of selected steroid substrates. The steroid-related tissue, including the reproductive and adrenal systems is characterized by high WWOX expression levels,^{20,21} which suggest that the WWOX protein can be involved in sex-steroid hormone metabolism through an SDR domain-containing conserved motifs typical of short-chain steroid dehydrogenases. Recombinant WWOX protein expressed in *E. coli* has been found to exert a redox activity on several steroid hormones.²⁰ As the majority of WWOX-related past research has been based on *in vitro* cell lines and *in vivo* animal studies, this review focuses on clinical cancer data.

WWOX in cancer

Changes in the WWOX gene chromosomal region and its expression have been observed in many cancers, including breast,²² ovarian,²³ prostate,²⁴ gastric,²⁵ and hepatocellular cancer.²⁶ Many articles report the presence of genetic alterations (mainly LOH—loss of heterozygosity) in WWOX and its reduced expression in many cancers (recently reviewed in Li *et al.*²⁷ and Gardenswartz and Aqeilan²⁸) but the clinical implications remain unclear. WWOX is thought to be a multifunctional protein whose specific action could be based on WW domains protein-protein differential interaction or the SDR domain metabolic activity, according to different protein partners, cell types, or tissue metabolic profiles. Despite the specificity of WWOX function, it has been shown that loss or reduction of its expression corresponds to a worse clinical prognosis in many cancers.^{29–32} The first studies reporting deletions in chromosome 16q in cancer tissue were published in the 1990s.^{33–35} In 1996, a detailed analysis of chromosomal markers revealed a high frequency of 16q23.3–16q24.1 LOH in ductal carcinoma *in situ* (DCIS).³⁶ The presence of such a high number of genomic alterations, which were found by Marcelo Aldaz's lab in almost 70% of non-invasive early stage breast cancers, detected in the region between the D16S515 and D16S504 markers, suggested that chromosomal abnormalities in this region could be the driving force of breast tumorigenesis. In 2000, Paige *et al.* mapped a homozygously deleted 700-kb region at 16q23.2 in ovarian cancer, as well as in several cancer cell lines, including colon and lung tissue.³⁷ The tumor suppressor gene WWOX was finally identified in the year 2000 and linked with the FRA16D common chromosomal fragile site.⁸

Breast cancer

As mentioned earlier, loss of WWOX heterozygosity was found in 70% or more of the DCIS samples.³⁶ WWOX expression was absent or reduced in about 60% of the DCIS and IDC (invasive ductal cancer) samples in contrast with normal human breast epithelium tissue.^{22,38} Interestingly, in breast cancer patients, greater WWOX expression was found to correlate with a higher apoptotic index (Bcl2/Bax ratio), while a low level of WWOX mRNA was found to correlate with lowered apoptosis and worse disease-free survival with significantly higher risk of disease recurrence (ratio = 3.48 [1.54–7.84]; $p = 0.039$).³⁹ This finding was confirmed by *in vitro* experiments using the MDA-MB-231 breast cancer cell line, in which ectopically elevated WWOX expression resulted in higher level of proapoptotic BAX protein and a decrease of antiapoptotic BCL-2, which also suggested that WWOX participates in the induction of apoptosis.³⁸ Furthermore, although the levels of procaspase-9 and procaspase-3 were depressed, the procaspase-8 level did not change. These results support the cancer suppressor theory and suggest that WWOX is associated with the mitochondrial apoptosis pathway.³⁸ Generally, breast cancer cell lines exhibit considerable diversity in WWOX expression, ranging from undetectable to high WWOX expression.²² By comparing changes in WWOX expression at the mRNA level, its expression was found to correlate negatively with patient age, i.e. expression thus is higher in malignant tissue from younger women. Moreover, the WWOX mRNA amount decreases with cancer advancement.³⁹ Based on *in vitro* experiments and clinical sample analysis, Aqeilan *et al.*¹³ propose that WWOX interaction with ERBB4 tyrosine kinase receptor, via the first WW domain, is essential for breast cancer development. WWOX physically binds to the cytoplasmic region of ERBB-4-containing PPXY motifs. Interaction between WWOX and ERBB4 in the cytoplasm prevented receptor translocation to the nucleus,^{13,19} thus counteracting breast cancer progression. Another report showed that WWOX expression was found to be absent in nearly all samples of metastatic breast tissue, together with a decreased level of cytoplasmic ERBB4.⁴⁰ Furthermore, reduced WWOX expression was found to correlate with a reduced amount of cytoplasmic ERBB4 in both metastatic tissues and a number of cancer-positive axillary lymph nodes.⁴⁰ Another member of the Her family whose expression was differentially disturbed in breast malignancies is ERBB3, which is responsible for initiating many cellular responses such as changes in gene expression upon activation of the AKT and MAPK signaling pathways,⁴¹ cytoskeletal rearrangement, antiapoptosis function,⁴² and a rise of cell proliferation.^{43,44} In samples of breast cancer tissue, loss of WWOX expression was correlated with the failure of ERBB3 and FHIT (tumor suppressor) proteins to localize in the cytoplasm.^{40,45,46} Unlike the classical tumor suppressor gene inactivation model, WWOX deletions/mutations of both alleles are rarely observed. Bednarek *et al.*⁴⁷ suggest that WWOX cellular function in cancer is affected by haploinsufficiency. Wang *et al.* note the presence of WWOX promoter methylation in 55% of breast cancer tissue samples,

but not in normal mammary tissue.⁴⁸ The cells of breast cancers in which the *WWOX* promoter was methylated exhibited a significantly greater reduction in expression of the *WWOX* gene than cells without promoter methylation. Another important feature is the existence of a positive correlation between estrogen receptor (ER) status and *WWOX* expression, could be a result of participation of the *WWOX* protein in sex-steroid metabolism.^{18,23} The *WWOX* protein has a SDR domain, which includes a conserved hormone-binding NSYK motif.⁴⁹ One *in vitro* study based on several breast cancer cell lines revealed that estradiol induces Tyr33 phosphorylation in the *WWOX* protein and its translocation to mitochondria.⁴⁹ *WWOX* expression was found to be a few times higher in ER-positive breast tumors than ER-negative ones, and also higher in progesterone-receptor positive breast tumors than negative ones.³⁹ Interestingly, it has been reported that while tamoxifen administration has no effect on disease-free survival for patients with both low *WWOX* expression and tumors positive for ERs, the efficiency of tamoxifen therapy was significantly higher for patients with cancers showing high levels of *WWOX* protein.⁵⁰ The level of *WWOX* expression has been found to vary depending on the stage of breast cancer. Chang *et al.* reported *WWOX* upregulation in the premetastatic stage but a significantly lower *WWOX* level in the metastatic stage.⁴⁹ Another group notes that *WWOX* expression is significantly lower in aggressive basal-like breast tumors compared to a non-basal-like subtype ($p=0.01$), and that lowered *WWOX* expression positively correlates with a worse disease-free survival (ratio=1.83; 95% CI, 1.01–3.28).⁵¹

Ovarian cancer

The role of the *WWOX* gene in hormone-dependent cancers was also investigated in ovarian tumors. Like breast cancer, *WWOX* expression was found to be lower in ovarian cancer,²³ a common female cancer with a high mortality rate.⁵² Nunez *et al.*⁵³ demonstrated lower expression in 40% of samples, and these were frequently associated with increased aggressiveness and less favorable outcomes. An *in vitro* study on the A2780 ovarian cancer cell line showed that *WWOX* overexpression resulted in reduced tumorigenicity and apoptosis induction.⁵⁴ Paige *et al.*⁵⁵ after examination of a cohort of 554 ovarian cancer cases reported that several *WWOX* gene single nucleotide polymorphisms (SNPs) were associated with disease-free survival, tumor grade, and histology.⁵⁵ However, these results differed from those obtained by a similar examination of another group of 863 cases, although this may be explained by populational differences or false positive associations in the previous study.⁵⁵ The progress of epithelial ovarian cancer is caused by both genetic and epigenetic changes, e.g. large deletions versus DNA methylation.^{52,56} In ovarian cancer tissue, the methylation of CpG islands was observed within the *WWOX* promoter, which was not detected in normal ovarian tissue.^{52,57} In addition, CpG methylation could be involved in ovarian cancer progression: *WWOX* promoter methylation was found to be markedly more frequent in the later stages (stage III and IV) of epithelial

ovarian cancer tissues than the early stages (stage I and II).⁵³ The same authors reported a statistically significant dependence between the loss of *WWOX* protein and shorter overall survival, as well as longer survival in cases which showed relatively high *WWOX* protein level.⁵³ A study of *WWOX* protein level in 112 epithelial ovarian carcinoma tissues revealed that loss of *WWOX* positively correlates with loss of ER and PR receptors; in addition, a statistically significant ($p=0.02$) reduction of *WWOX* protein level was also observed in ovarian cancers in advanced stages of disease, according to the International Federation of Gynecology and Obstetrics staging, which could be of clinical importance.⁵⁸

Prostate cancer

The most common hormone-dependent cancer in men is prostatic neoplasm. It was reported that in approximately 70% cases of prostate cancer, a reduced or undetectable level of *WWOX* expression can be found, which is caused by deletion or epigenetic modifications.^{24,59} The *WWOX* promoter and exon 1 were hypermethylated in prostate cancer cells, compared to normal prostate cells.⁵⁹ Similarly to breast and ovarian cancers, *WWOX* overexpression induced apoptosis and inhibited cancer cell growth *in vitro* and *in vivo*.²⁴ *WWOX* activated apoptosis through the caspase pathway or by binding to the p73 protein.²³ According to Suarez *et al.*,⁶⁰ a wide genome scan performed on a cohort of brothers with prostate cancer identified strong association between prostate cancer and chromosomal marker D16S3096 (localized in intron 8). A similar linkage between prostate cancer and another marker, rs1079635, was observed by Lange *et al.*⁶¹ in a group of 131 Caucasian prostate cancer families.

Liver cancer

Although liver cancer is another type of epithelial cancer, it has no direct hormonal component as breast, ovary, and prostate cancers. Hepatocellular carcinoma (HCC) has been used as the subject of a number of studies concerned with the suppressor role of *WWOX* in tumor development. Liver cancer is the sixth most common malignant cancer in the world, with its most common occurrences being found in Southeast Asia and South Africa, where hepatitis B virus (HBV) infection is extremely prevalent⁶²; HBV infection being thought to be the major factor causing liver cancer.^{26,63} Other risk factors include chronic hepatitis C virus infection, food contaminated with aflatoxin,⁶² cirrhosis as a result of alcohol abuse,⁶⁴ or inherited metabolic disorders.⁶⁵ In addition, tobacco smoking, obesity, diabetes,⁶² and parasitic infection⁶⁶ are other probable causes. Liver cancer is highly invasive and difficult to treat, resulting in a high mortality rate.^{64,67,68} This type of cancer is often diagnosed at an advanced stage and survival outcome depends on other coexisting liver disturbances, for example, the chance of survival is poorer when the patient also suffers from hepatitis or cirrhosis.^{69,70} The most common histological type of liver cancer is HCC, which develops from the glandular cells lining the liver.⁶² The relative five-year

survival of HCC patients is only 15%.⁶⁹ The presence of the risk factors given above contributes to a state of chronic inflammation in the liver, with the result that the normal cell environment is converted into a mutagenic one: a situation which favors an accumulation of genetic alterations.⁶² For instance, the common fragile site FRA16D is often altered in HCCs. This site is quite susceptible to damage inducing by various carcinogens, which explains the high frequency of *WWOX* gene alterations in HCC. As already mentioned, this type of cancer is closely associated with exposure to chemical carcinogens and infection with oncogenic viruses.⁶² As the expression of the *WWOX* gene has been found to be weak or undetectable in about 50–75% cases of hepatocellular cancer tissues, *WWOX* is believed to be involved in HCC tumorigenesis.^{26,71} Additionally, it has been proposed that lower *WWOX* levels correlate with poorer clinicopathological features,²⁶ which is consistent with the findings of studies on hepatocellular cancer cell lines.^{67,71} From a study based on a panel of 18 HCC cell lines, Park *et al.*⁶⁷ reported a decrease or absence of *WWOX* mRNA in about 60% and a decrease of *WWOX* protein in about 75% of the cell lines, in comparison to normal liver. However, it is difficult to determine whether *WWOX* gene alterations in HCC are a cause or an effect of liver tumorigenesis, since the majority of cell lines have unbalanced translocations within chromosome 16, particularly in the *WWOX* locus, where some breakpoint occurred. Findings from another cell line study reveal that downregulation of *WWOX* expression is supposed not to be related to HBV infection, with five out of six examined HBV-negative cell lines demonstrating low *WWOX* expression.⁶⁷ Moreover, suppression of *WWOX* expression in liver cancer seems not to be a result of promoter methylation or point mutations since they have rarely been found in HCC. Aderca *et al.*⁷¹ suggest that the *WWOX* gene promoter is not regulated by methylation in HCC cell lines, as seen in breast cancer cell lines, as increased *WWOX* expression was not observed after treatment with the demethylating agent 5-aza-2'-deoxycytidine.⁷¹ However, the same authors reported the presence of *WWOX* gene promoter methylation in some primary HCC samples with low *WWOX* levels.⁶² It has been suggested that lack of *WWOX* is involved in the development and differentiation of HCC.²⁶ A recent comparison of *WWOX* expression in HCC tissue with that of adjacent non-tumor tissues revealed a correlation between the downregulation of *WWOX* and a higher histological grade in HCC tissues.²⁶

Osteosarcoma (OS)(bone tumors)

Bone tumors are comparatively scarce forms of cancer.⁷² They can be classified into three groups: tumors with specific chromosomal translocations (Ewing sarcoma, aneurysmal bone cyst), bone tumors characterized by specific gene mutations or amplifications (chondrosarcoma, fibrous dysplasia, chordoma), and cancer with genetic instability (OS).⁷³ OS is the most common bone malignancy among children and adults over 65 years old,⁷⁴ comprising 20% of all bone cancers and 5% of children's cancers.⁷⁵ The OS develops from a mesenchymal stem cell as a consequence of

genetic and epigenetic interruptions of osteoblast differentiation pathways.^{74,75} It is also caused by such environmental factors as ionizing radiation (responsible for 3% of cases),⁷⁶ alkylating factors,⁷⁷ and perinatal factors (short birth length, high birth weight).⁷⁸ Some proteins such as p53, pRB, RUNX2, DVL-2, and EZRIN play a role in the genetic interruption of OS cells. Yang *et al.*⁷⁹ reported lowered *WWOX* expression in human OS: *WWOX* genomic alterations were found in three out of 10 frozen samples while undetectable *WWOX* protein levels were found in 34 out of 55 paraffin-fixed OS samples. Their results suggest that the absence of *WWOX* protein, caused by gene deletion or loss of protein expression, is likely an early event in OS pathogenesis.⁷⁹ Similar results were obtained by Kurek *et al.*,⁸⁰ who found *WWOX* protein levels to be lower in 48 of 83 OS samples from 51 patients, and that seven out of 16 paired post-treatment samples from patients showing a poor response to chemotherapy were found to have higher *WWOX* protein levels than the corresponding primary tumor tissue samples. They also found *WWOX* protein levels in four out of nine metastatic OS samples to be below those of primary tumor samples.⁸⁰ Complementary results showing the influence of reduced *WWOX* expression on mRNA level were reported by Diniz *et al.*⁸¹ in several different bone lesions: two OSs, two fibrosarcoma, and eight ossifying fibroma. Yang *et al.*⁷⁹ reported the presence of deletions in the *WWOX* gene region in six out of 10 OS samples based on comparative genomic hybridization analysis, and undetectable levels of *WWOX* protein in 61.1% (33/54) of OSs, based on immunohistochemistry staining.⁷⁹ Strong molecular evidence of *WWOX* involvement in bone tissue differentiation and bone carcinogenesis has been reported by cell line and knock-out mouse studies. Aqeilan *et al.*¹⁸ report that *WWOX* directly interacts with RUNX2 by the first WW domain, thus suppressing RUNX2 function and reducing its expression. An elevated level of RUNX2 was detected in *Wwox*^{-/-} mouse femurs.⁸² Simultaneously, expression of osteoblast differentiation markers in *Wwox*^{-/-} femurs, i.e. alkaline phosphatase, collagen type I, bone sialoprotein, and osteocalcin, increased about 50%.¹⁸ In knockout mice, *Wwox* ablation led to osteoblast deregulation and their conversion to OS-like cells.⁸³ A study conducted on *Wwox* knockout mice demonstrated some imperfections in bone metabolism: their bones were characterized by smaller size,¹⁸ decreased density,¹⁸ and less mineralization,⁸⁴ and about 30% of juvenile mice developed OSs.¹⁸ Furthermore, most OS cell lines demonstrated lowered *WWOX* levels. In addition, *WWOX* overexpression caused reduced *in vitro* tumorigenicity through increased apoptosis and depressed proliferation.⁸⁰ *WWOX* was also found to interact with EZRIN, ERBB4, and DVL-2 proteins. The first two are related with OS development,⁸² while DVL-2 engages in osteoblast differentiation.⁸⁵ As mentioned earlier, *WWOX* is an inhibitor of the Wnt/B-catenin pathway. The Wnt/B-catenin pathway was found to be inactive in 90% of clinical OS samples because B-catenin had not achieved nuclear localization.⁸⁶ Another important protein is EZRIN, whose expression is observed in about 60% of OS patients.⁸⁷ Disease recurrence was seen to be more common in OS patients with elevated *EZRIN*

expression and overall survival after tumor resection was shorter.⁸⁷ Moreover, higher *EZRIN* expression was detected in high-grade OSs compared to low-grade OSs.⁸⁸ However, it was proposed that by interacting with *EZRIN*, *WWOX* is able to control this protein and moderate the process of OS metastasis.⁸⁹ There is an interesting *in vitro* study showing that ectopically elevated *WWOX* sensitizes OS SaOS2 cells to cisplatin treatment.⁹⁰

Tumors of central nervous system

Tumors of the central nervous system are, in fact, a set of tumors characterized by a specific molecular cell biology, but with different prognoses, survival rates, and therapeutic approaches. They constitute less than 2% of all cancers.⁹¹ About 50% of all intrinsic brain tumors are gliomas, which is not surprising, since glia is tissue which surrounds and supports neurons in the central nervous system and significantly, glial cells preserve their ability to proliferate.⁶⁵ The most common tumor derived from glial tissue is astrocytoma, which has been classified into four histological grades by the World Health Organization with respect to degree of malignancy.⁹² Two tumors which are extremely malignant and widespread in adults are anaplastic astrocytoma and glioblastoma multiforme (GBM), which are classified as grade III and IV, respectively.^{65,92} Glioblastoma can be further classified as either primary or secondary. Primary GBM is the most common and most malignant type of glia tumor, which in bulk cases (50–60%) affects adults older than 50 years.⁹³ Glioblastomas usually develop in the cerebral hemispheres but are able to spread into the basal ganglia.⁹³ This kind of cancer is characterized by a high lethality, exceedingly rapid infiltrative growth, and a short period of overall survival of, more or less, 14 months, despite aggressive therapy in the form of maximal surgical resection combined with radiotherapy and temozolomide chemotherapy.^{94–96} Moreover, the existence of the blood-brain barrier, whose role is to protect the central nervous system against deleterious agents, including chemotherapeutics, significantly hampers treatment. Additionally, the low competence of brain tissue to regenerate combined with the ability of GBM cells to invade normal brain tissue causes therapy to be extremely troublesome.^{94,97} Recent studies have addressed the role of the *WWOX* suppressor gene in tumorigenesis and tumor progression of GBM and other glia tumors.^{92,94,97} In one analysis of tissue samples from patients with GBM, Kosla *et al.*⁹⁷ examined the state of promoter methylation and the relationship between the expression of *WWOX* and genes such as *KI67*, *EGFR* and *BAX*, *BCL2*. They found only sporadic methylation of the *WWOX* gene promoter (15–18%), which was associated with a reduction of *WWOX* mRNA level.⁹⁷ Winardi *et al.*⁹² observed a significantly decreased expression of the *WWOX* gene in patients with various grades of astrocytoma, which correlated with patient age, supratentorial localization of the tumor, and severity of the symptoms, but not with tumor grade.⁹² Yu *et al.*,⁹⁸ in a study performed on a large cohort of 1798 glioma patients and 1824 healthy controls, found the deletion of copy number variants of CNV-67084, representing the *WWOX* genomic

region, to be significantly associated with risk of glioma. This study also revealed a significant reduction of *WWOX* mRNA in glioma samples compared with adjacent tissues.⁹⁸ A histoimmunochemical study of a number of brain tumors by Chiang *et al.*⁹⁹ revealed that the *WWOX* protein level in normal cortical neurons was lower than in microcystic and malignant meningiomas and in the meningotheomatous area. Interestingly, two functional missense mutations of *WWOX* were reported in non-cancerous brain diseases including tonic-clonic epilepsy, mental retardation and ataxia, as well as in upper motor neuron disease.^{100,101}

Hematopoietic malignancies

Hematological cancers develop from cells of the hematopoietic lineage. These cancers are classified according to the type of cell from which the cancer is derived and its stage of development, defined by morphology and protein markers. According to this classification, the simplest division of hematological cancers comprises leukemias and lymphomas. To better understand the role of the *WWOX* gene in hematopoietic disorders, Ishii *et al.*¹⁰² analyzed samples from 74 patients with various primary hematopoietic neoplasias and 20 leukemia-derived cell lines in respect of expression of *WWOX* mRNA.¹⁰² Their studies revealed that 51% of the samples from patients and 55% of leukemic cell lines displayed aberrant or lack of *WWOX* expression. The analysis of aberrant *WWOX* transcripts revealed that an incomplete set of exons were expressed in acute myelogenous leukemia (AML-M4), acute lymphoblastic leukemias (ALL-L1, ALL-L2), and the KCL22 cell line.¹⁰² Additionally, PCR amplification of the products of each coding exon of the *WWOX* gene expressed by the three cell lines KG1, U937, and HEL, as well as in peripheral lymphocyte culture derived from a healthy volunteer, revealed four nucleotide variations in the *WWOX* gene.¹⁰² In U937, C-to-T substitution was observed at nucleotide 357 of the cDNA, while A-to-T substitution at nucleotide 805 was observed in HEL, and a guanine residue was found to be inserted at position 706 of in KG1 cell line. However, as these cell lines have no normal equivalent, it is not clear whether the described variants are polymorphisms or point mutations. One recently described polymorphism of the *WWOX* gene is based on the transversion of a cytosine to a guanine at base position 844 in the coding sequence.^{102,103} Additionally, chromatin was found to exert an influence on *WWOX* expression by histone methylation and acetylation. The K562 cell line, characterized by almost undetectable level of *WWOX* mRNA, was grown in medium supplemented with inhibitors of methylation and deacetylation, resulting in an elevated number of aberrant and wild-type *WWOX* transcripts being observed.¹⁰² The same studies addressed the role of multiple fragile genes. The findings suggest that the expression of *WWOX* and *FHIT* genes at two of the most active common fragile sites is associated with hematopoietic malignancies.^{102,104} Although the transcripts of both genes were expressed equally, *WWOX* gene expression was reduced to 51% of the normal value while *FHIT* was reduced to 36%,^{102,104} which implies that the

Table 1 Cancer clinical factors associated with aberrations of *WWOX*

Cancer type	Clinical significance	<i>WWOX</i> prevalent alterations	References
Breast	Reduced <i>WWOX</i> expression is associated with worse disease-free survival with significantly higher risk of disease recurrence and worse efficiency of tamoxifen administration in patients	Loss or reduction of the expression Loss of heterozygosity (LOH>70%) and sporadic promoter methylation A significantly lower expression in ER-negative versus ER-positive breast tumors	22, 36, 38 48 39, 50
Ovarian	Lower <i>WWOX</i> expression is associated with increased aggressiveness and less favorable outcomes Disease-free survival, tumor grade, and histology are accompanied with changes of single nucleotides	Loss or reduction of the expression Promoter methylation Functional SNPs within gene	23, 53 52, 53, 57 55
Prostate	A suggested role of <i>WWOX</i> in prostate cancer development	Loss or reduction of the expression Hypermethylation of promoter and exon 1	24, 59 59
Liver	Reduction of <i>WWOX</i> expression correlates with poorer clinicopathological features	Loss or reduction of the expression	26, 71
Gastric	Putative contribution of <i>WWOX</i> gene in gastric tumorigenesis	Loss of heterozygosity (LOH) Loss or reduction of the expression	25 25
Osteosarcoma	<i>WWOX</i> level is associated with pathogenesis and aggressiveness of osteosarcoma	Loss or reduction of the expression Reduced protein level in some metastatic osteosarcoma samples Increased protein level after chemotherapy	79–81 80 80
Central nervous system	It is presumed that decreased <i>WWOX</i> expression is correlated with severity of the symptoms	Sporadic promoter methylation Loss or reduction of the expression	97 98, 99
Hematopoietic malignancies	A suggested role for <i>WWOX</i> in various primary hematopoietic neoplasias	Loss or reduction of the expression Promoter methylation	102, 107 106
Childhood cancers	Lower level of <i>WWOX</i> expression is associated with less favorable prognosis The occurrence of LOH is associated with higher disease recurrence, metastasis, and mortality	Loss or reduction of the expression Promoter methylation Loss of heterozygosity (LOH)	109, 110 110 110

expression of the mutated *WWOX* and *FHIT* genes might be a consequence of such epigenetic modifications as the operation of splicing machinery in proliferating tumor cells rather than large deletions.^{102,104,105} Chen *et al.*¹⁰⁶ found the mRNA expression and methylation status of *WWOX*, *FHIT*, and *p73* genes to be significantly lower in all ALL cases than controls. Loss of mRNA expression was found in approximately 51% of cases for *WWOX* and 57% for *FHIT* indicated that altered *WWOX* gene expression accompanies altered *FHIT* gene expression in ALL, which leads the authors to suggest that concordant expression of these genes has an influence on the progression of ALL. Both Ishii *et al.*¹⁰² and Chen *et al.*¹⁰⁶ postulate that concordant expression of both *WWOX* and *FHIT* genes have an influence on the progression of ALL and other hematopoietic neoplasms. However, while Chen reports that the expression of *WWOX* and *FHIT* was altered to similar degrees, Ishii notes otherwise.¹⁰⁴ These differences may be explained by the composition of the groups of patients: the first was composed of patients with ALL while the second was made up of patients with various hematopoietic disorders. In addition, the two studies addressed the changes of

WWOX level and *p73* expression during apoptosis in ALL. The studies showed that the expression profile of *WWOX* was similar to that of *p73*, and both were significantly lower than controls, indicating that the *WWOX* expression positively correlates with *p73* expression. Hence, reduction of *WWOX* expression might suppress the apoptotic role of *p73*, and thereby contribute to the occurrence and progression of ALL.¹⁰⁶ Chen *et al.*¹⁰⁶ also examined the influence of methylation status on the inactivation of *WWOX*, *FHIT*, and *p73* genes in ALL.¹⁰⁶ Approximately 40% of the *WWOX* and the *FHIT* promoter regions and the first exon of the *p73* gene were methylated, which contrasted with control samples, where methylation was not noticed in corresponding regions. The authors postulate that the highly methylated status of the three genes contributed to their silencing due to the lack of transcription products, which was reflected in progression of ALL. Nevertheless, they did not confirm any correlation between the frequency of *WWOX* methylation and *FHIT* or *p73* expression or methylation.¹⁰⁶ In addition, Cui *et al.*¹⁰⁷ report a reduced level of *WWOX* mRNA and *WWOX* protein in leukemia patients.

Childhood cancers

Deletions of the chromosome 16q arm have been reported in Wilms' (nephroblastoma) tumors in dozens of scientific reports. However, in 2000,¹⁰⁸ aberrations in the FRA16D chromosomal region occupied by WWOX were reported, showing a greater than 21% LOH to be associated with higher disease recurrence, metastasis, and mortality. Real-time RT-PCR revealed aberrant WWOX expression in two early age cancers: neuroblastoma¹⁰⁹ and Wilms' tumor.¹¹⁰ Hemizygoty of D16S3096 genomic marker was observed in 50% of neuroblastoma and 30.4% of nephroblastoma tumors while observed populational homozygoty is around 26%.^{109,110} Interestingly, a higher level of WWOX expression was associated with a favorable prognostic category according to the International Neuroblastoma Pathology Classification.¹⁰⁹ Similarly, LOH was observed in intron 8 (D16S3096) in Wilms' tumor samples. Moreover, significant reduction of WWOX mRNA expression was found to be associated with promoter methylation in 13.64% of nephroblastoma samples.¹¹⁰

Summary

Despite of questions whether WWOX is a driver or passenger of carcinogenesis or progression of disease, there is a growing evidence showing associations of WWOX alterations with tumor type, stage, and aggressiveness (Table 1). Evidence from cell lines and animal models support its tumor suppressor activity in many cancers. For most tumors haploinsufficiency as a result of a single allele deletion is a prevalent way of WWOX inactivation. However, some evidence of its promoter methylation, SNP and mutations were reported in a number of cancer cases. Several transcription factors were identified as WWOX partners suggesting distinct behavior in different tissues and cell types. However, silencing of WWOX expression is associated with more advanced tumors, higher aggressiveness, and poorer disease-free and overall patients' survival in majority of studied cancer types. WWOX is not a classical tumor suppressor gene due to the fact that inactivation of both alleles and missense mutations is a very rare event. Several reports have shown that WWOX expression evaluation using real-time RT-PCR immunohistochemistry could be a useful diagnostic-prognostic test that can be used not only in a prognosis of a progress of cancer diseases but also prediction of therapy effectiveness.

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