PROSPECT
Journal of Cellular Biochemistry 108:737–745 (2009)

Journal of Cellular Biochemistry

WWOX: Its Genomics, Partners, and Functions

Sara Del Mare, Zaidoun Salah, and Rami I. Aqeilan*

The Lautenberg Center for General and Tumor Immunology, Institute for Medical Research Israel-Canada, The Hebrew University–Hadassah Medical School, Jerusalem 91120, Israel

ABSTRACT

The WW domain-containing oxidoreductase (*WWOX*) spans one of the most active common fragile sites (CFSs) involved in cancer, FRA16D. *WWOX* encodes a 46-kDa protein that contains two N-terminal WW domains and a central short-chain dehydrogenase/reductase (SDR) domain. Through its WW domain, Wwox interacts with its partners and modulates their functions. Our data indicate that Wwox suppresses the transactivation function of several transcription factors implied in neoplasia by sequestering them in the cytoplasm. Work from our laboratory and other research groups have demonstrated that Wwox participates in a number of cellular processes including growth, differentiation, apoptosis, and tumor suppression. Targeted deletion of the *Wwox* gene in mice causes increased spontaneous and chemically induced tumor incidence supporting *bona fide* tumor suppressor function of *WWOX*. Moreover, generation of the *Wwox*-deficient mice uncovers, at least in part, some of the physiological in vivo functions of the *WWOX* gene. This review focuses on recent progress that elucidates Wwox functions in biology and pathology. J. Cell. Biochem. 108: 737–745, 2009.

KEY WORDS: WWOX; TUMOR SUPPRESSOR; WW DOMAINS; PROTEIN–PROTEIN INTERACTION; COMMON FRAGILE SITES

Malignant transformation is a complex multistep process that consists of different combinations of genetic alterations. The genome of an early transforming cell acquires activated alleles of oncogenes, mutated or lost tumor suppressor genes, and alterations of other genes responsible for genome stability [Hanahan and Weinberg, 2000]. These combinations of genetic abnormalities generate cells that divide more rapidly or evade cell death, thus, liberating them from growth control and cell cycle checkpoints. Mammalian cells have multiple safeguards to protect them against these genetic changes and only when several genes are defective does a cancer develop. Thus, cancer is the result of a multistep process involving sequential genetic alterations. The initiating genetic alterations are extremely important to uncover since development of malignancy depends on these specific genetic alterations. Targeting the initiating event should be therefore among the first priorities in the development of rational therapies. Other genetic alterations take place during tumor progression which likely result in heterogeneity of tumors. This tumor heterogeneity has a great clinical impact due to the differences in clinical behavior and responses to treatment of tumors of the same diagnostic type.

Most common epithelial tumors such as breast, prostate, stomach, and others are often characterized by homozygous deletion and loss of heterozygosity (LOH) involving the long arm of chromosome 16 (16q) [Chen et al., 1996; Latil et al., 1997; Yu et al., 1997]. In addition, the chromosomal region 16q23.2 contains one of the most common human fragile sites, FRA16D, and four translocation breakpoints associated with multiple myeloma [Chesi et al., 1998; Mangelsdorf et al., 2000; Paige et al., 2000]. Therefore, it has been suggested that a tumor suppressor gene is located in this region. In 2000, the WW domain-containing oxidoreductase (*WWOX*) gene, also known as fragile site FRA16D oxidoreductase (*FOR*), was cloned and found to span FRA16D [Bednarek et al., 2000; Ried et al., 2000]. Genomic analysis reveals that *WWOX* is a very large gene spanning ~1.11 Mb consisting of nine small exons separated by large introns (Fig. 1). Alternative splicing of *WWOX* generates transcript variants that encode seven different isoforms containing two WW domains and a central short-chain dehydrogenase/reductase (SDR). The predominant and ubiquitously expressed full-length *WWOX* transcript variant is ~1.2 kb and codes for a 414 amino acids that form a protein of 46.6 kDa [Bednarek et al., 2000; Ried et al., 2000]. Subsequent studies demonstrate that *WWOX* expression is lost or reduced in a variety of human malignancies making *WWOX* the fragile gene target of genomic alterations at 16q23.2. Since 2000, worldwide interest in the *WWOX* gene has grown steadily, a trend reflected in the increase in publication on the topic, which number more than 130 to date.

Grant sponsor: EU-FP7 Marie-Curie Re-integration Grant; Grant sponsor: Israel Cancer Research Fund (ICRF); Grant sponsor: Israel Science Foundation (ISF).

*Correspondence to: Dr. Rami I. Aqeilan, The Lautenberg Center for General and Tumor Immunology, The Hebrew University–Hadassah Medical School, Jerusalem 91120, Israel. E-mail: aqeilan@cc.huji.ac.il

Received 6 July 2009; Accepted 7 July 2009 • DOI 10.1002/jcb.22298 • © 2009 Wiley-Liss, Inc.
Published online 25 August 2009 in Wiley InterScience (www.interscience.wiley.com).

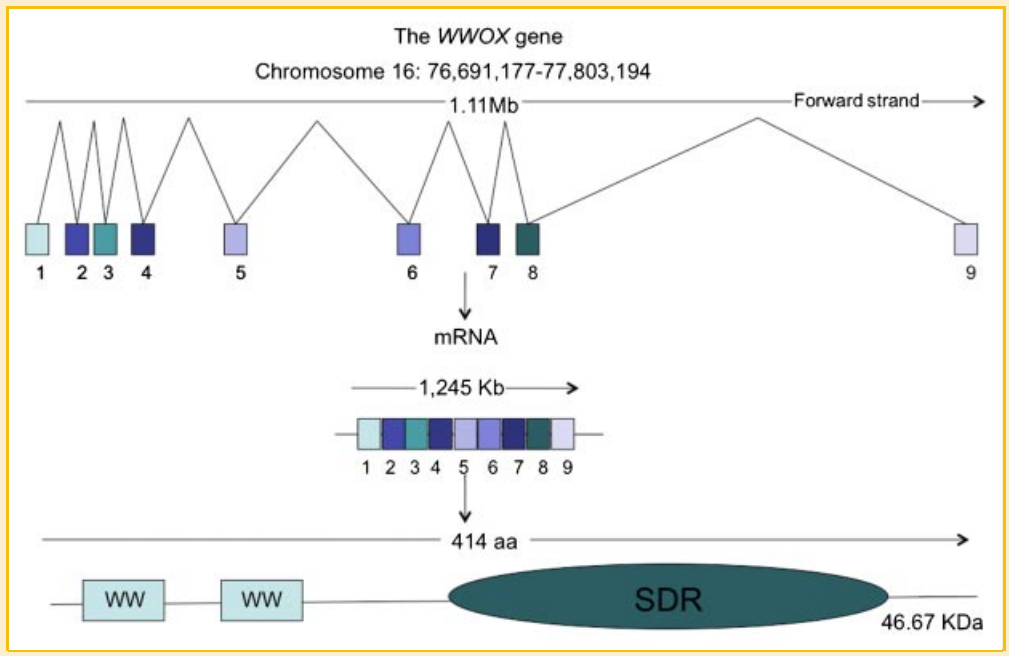

Fig. 1. The *WWOX* gene and its gene product. The human *WWOX* gene maps to chromosome 16q23 and spans 1.1 Mb. The full-length *WWOX* contains nine exons coding for 1,245 bp mRNA transcript that encodes for 414 amino acids. The protein contains two N-terminal WW domains and a central short-chain dehydrogenase/reductase domain (SDR). Alternative splicing of *WWOX* mRNA generates seven transcripts, though protein isoforms are not well documented. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

LOSS OF *WWOX* IN MOST COMMON HUMAN CANCERS

The region that the *WWOX* gene spans has attracted the interest of several research groups due to the fact that it contains a common fragile site (CFS) [Mangelsdorf et al., 2000; Ried et al., 2000]. CFSs are large genomic regions that are susceptible to chromosomal translocations, rearrangements, sister chromatid exchange and to site-specific gaps or breaks in metaphase chromosomes [Glover, 2006]. Expression of CFS occurs under conditions that inhibit DNA replication such as treatment with chemical agents that interfere with DNA synthesis such as aphidicolin [Glover, 1998]. In fact, fragile sites are demonstrated to be regions with a high density of Alu repeats, in which DNA replication is delayed and/or markedly slowed down. Failure to complete replication of CFS sequences before condensation of chromatin into metaphases chromosomes may give rise to the chromosomal breaks and gaps characteristic of fragile sites [Palakodeti et al., 2004; Glover, 2006]. Recently, the second most highly expressed CFS in human cells, FRA16D, has been mapped to the 16q23.2 region, coinciding with the region of homozygous deletions found in various malignancies [Mangelsdorf et al., 2000; Paige et al., 2000]. The region of instability is located within the large intron eight spanning ~750 kb of the *WWOX* gene [Ried et al., 2000] (Fig. 1).

Loss of *WWOX* expression has been identified in a variety of tumors including breast, esophageal, lung, ovarian, colon, prostate, and gastric carcinomas [Bednarek et al., 2000; Paige et al., 2001; Driouch et al., 2002; Kuroki et al., 2002; Yendamuri et al., 2003; Aqeilan et al., 2004a]. In one study, Driouch et al. [2002] reported *WWOX* truncated variants in clinical breast cancer specimens as well as in cell lines. These forms contained deleted exons 6,7, and 8 corresponding to the SDR domain of *WWOX*. Aberrant transcripts missing exons 5,6,7, and 8 were also detected in lung and gastric carcinoma cell lines [Yendamuri et al., 2003; Aqeilan et al., 2004a]. Although point mutations are not detected, LOH is common in almost one-third of tumor samples analyzed [reviewed in Aqeilan and Croce, 2007]. Moreover, biallelic deletions, resulting in homozygous loss of FRA16D sequences, have been detected in adenocarcinomas of stomach, colon, lung, and ovary [Paige et al., 2000; Ried et al., 2000; Finnis et al., 2005]. Altogether, these data imply that *WWOX*, spanning the second most-active CFS, is the target of genomic aberration at 16q23.2, and that its loss is a common event in cancer development.

Presumably, the genomic location of *WWOX* in CFS makes it susceptible to breakage, to LOH, and to homozygous deletions when exposed to carcinogens (Fig. 2). Thus, *WWOX* alterations could also be common in cancers caused primarily by carcinogen exposure. This is supported by evidence that *WWOX* alterations are common in esophageal [Kuroki et al., 2002] and lung carcinomas [Yendamuri et al., 2003; Donati et al., 2007], both of which are associated with tobacco and alcohol use. Additionally, *WWOX* expression is altered in gastric carcinoma [Aqeilan et al., 2004a] that is strongly associated with exposure to environmental and dietary carcinogens. Generally, carcinogens can cause DNA damage that can lead to inactivation of tumor suppressor genes, such as *WWOX*, that may have a role in DNA repair processes. Therefore, its loss could

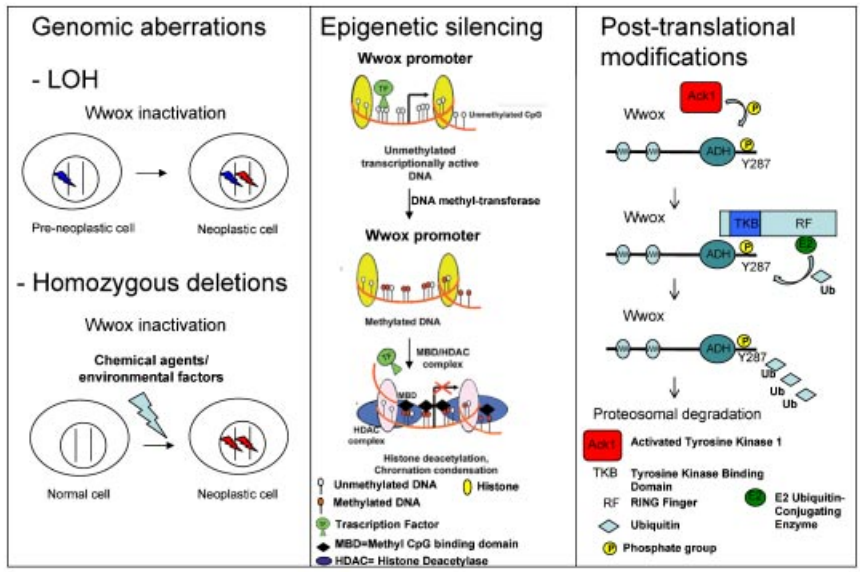

Fig. 2. Regulation of *WWOX* expression in cancer cells. *WWOX* alterations in human cancer could be due to genomic, epigenetic and post-translational modifications. Due to its location in FRA16D, the second most active common fragile site, *WWOX* is susceptible to genomic aberrations such as LOH or homozygous deletions, resulting in gene inactivation. In many cancer types, Wwox loss is associated with promoter hypermethylation that leads to chromatin condensation and gene silencing. Finally, Wwox protein can be degraded due to polyubiquitination by Ack1 tyrosine kinase. Ack1 phosphorylates Wwox on tyrosine 287, which promotes its degradation.

promote progressive accumulation of mutations or other genetic alterations that then lead to tumor initiation and progression. Intriguingly, a statistically significant correlation between the expression of Wwox and Fhit protein is reported in cancer generally [Aqeilan et al., 2004a; Guler et al., 2005]. The *FHIT* gene encompasses the most active CFS, FRA3B [Ohta et al., 1996], and is sensitive to intragenic alterations by DNA damaging agents [Huebner and Croce, 2003]. This can lead to allelic loss in the preneoplasia that coincides with activation of a DNA damage checkpoint [Bartkova et al., 2005; Gorgoulis et al., 2005]. This suggests that alteration of CFSs takes place in an early stage of cancer transformation, thus, leading to the loss of expression of genes localizing within their boundaries, such as *WWOX* and *FHIT*.

Other mechanisms including epigenetic and post-translational modifications have also been demonstrated to be responsible for the regulation of *WWOX* levels in cancer cells (Fig. 2). Hypermethylation-mediating silencing of *WWOX* has been reported in several types of cancer including pancreatic [Kuroki et al., 2004], breast, and lung carcinomas [Iliopoulos et al., 2005]. Furthermore, restoration of *WWOX* expression using epigenetic modulation approaches suppresses tumorigenecity both in vitro and in vivo [Cantor et al., 2007]. Another mechanism possibly regulating Wwox protein levels in cancer cells is Wwox polyubiquitination. Mahajan et al. [2005] have demonstrated that Ack1promotes Wwox polyubiquitination, thus, leading to its degradation. Ack1 is a tyrosine kinase that is upregulated in prostate cancer cells; therefore, its overexpression can lead to downregulation of Wwox in prostate cancer [Qin et al., 2006]. These data indicate that loss of Wwox expression in cancer cells could be due to genomic, epigenetic, and post-translational modifications (Fig. 2).

TUMOR SUPPRESSOR ACTIVITY OF *WWOX*

The deregulation of *WWOX* expression in human cancer is a common event [O'Keefe and Richards, 2006; Aqeilan and Croce, 2007] suggesting that *WWOX* functions as a tumor suppressor. In an attempt to explore the tumor suppressor behavior of *WWOX*, Bednarek et al. [2001], have shown that restoration of *WWOX* in breast cancer cells lines, harboring low expression of endogenous *WWOX*, results in inhibition of anchorage-independent growth and suppression of tumorigenecity in vivo. In a subsequent study, Fabbri et al. [2005] demonstrate that viral-mediated overexpression of *WWOX* in lung cancer cell lines enhances apoptosis mediated by caspase-3 activation and leads to inhibition of tumorigenecity in vivo. Overexpression experiments in breast [Iliopoulos et al., 2007], prostate [Qin et al., 2006], and pancreatic [Nakayama et al., 2008] cancer cells also support tumor suppressor function of *WWOX*. These findings also suggest that loss of Wwox expression is associated with a growth advantage of cancer cells and that restoration of *WWOX* in these cells sensitizes them to apoptosis. However, these data do not provide direct evidence for the association of Wwox with tumor suppressor function.

An important step in the functional characterization of a given gene is the generation of animal models that resemble its alteration in human cancers. Since *WWOX* is inactivated in most human cancers and exhibits tumor suppressor activity in various cell lines, a *Wwox* knockout mouse can provide a good tool for studying Wwox anti-tumor function. The murine *Wwox* gene is similar to its human homologue; it spans a CFS *Fra8E1* [Krummel et al., 2002] and induces apoptosis in mouse cell lines [Chang et al., 2001]. Therefore, the mouse *Wwox* gene is an appropriate model for studying the

tumor suppressor function of the human *WWOX* gene. In 2007, we developed a mouse strain lacking *Wwox* expression [Aqeilan et al., 2007c]. Targeted ablation of the murine *Wwox* gene led to post-natal lethality thus precluding adult tumor analysis. Nevertheless, juvenile mice, by age of 3 weeks, developed focal lesions along the diaphysis of their femurs resembling early osteosarcomas. More than 30% of *Wwox* null mice develop periosteal osteosarcoma. Unfortunately, it was not possible to follow the progression of such tumors due to post-natal lethality. Nevertheless, biochemical analysis of Wwox partners reveals that physical and functional association of Wwox with the master transcription factor specific for osteoblast differentiation, Runx2, might be responsible for the development of osteosarcoma in *Wwox*-deficient mice (see below).

In order to examine whether inactivation of one allele of the *Wwox* gene contributes to tumorigenesis, we also monitored *Wwox*-heterozygous ($Wwox^{+/-}$) mice for spontaneous tumor development [Aqeilan et al., 2007c]. $Wwox^{+/-}$ and wild-type ($Wwox^{+/+}$) matched-littermate mice were monitored for 2 years. We observed that the incidence of tumor formation in $Wwox^{+/-}$ mice is significantly higher than in $Wwox^{+/+}$ mice. The spontaneous tumors in $Wwox^{+/-}$ mice included lung and mammary tumors [Aqeilan et al., 2007c; and unpublished data]. Interestingly, the second *Wwox* allele in some cases was intact, suggesting haploinsufficient features of *WWOX*. These tumors appeared in aging mice indicating that loss of one allele of *WWOX* may contribute to the evolution of other genetic alterations that lead to tumor formation. Our findings indicate that inactivation of both *WWOX* alleles results in osteosarcomas while inactivation of one allele contributes to lung and mammary carcinomas.

To define the role of *WWOX* in tumor progression, $Wwox^{+/-}$ and $Wwox^{+/+}$ mice were treated with chemical carcinogens and the incidence of tumor formation was evaluated. In one study we utilized the chemical mutagen ethyl-nitros urea (ENU) [Aqeilan et al., 2007c]. Forty weeks after administration of ENU, incidence of tumor formation in $Wwox^{+/-}$ mice was 80% whereas it was half of that in $Wwox^{+/+}$ mice. The tumor spectrum included lymphoblastic leukemia as well as lung, mammary, and liver tumors. In a second study, $Wwox^{+/-}$ mice were treated with the established esophageal/forestomach carcinogen *N*-nitrosomethyl-benzylamine (NMBA), and the frequency of tumor formation in these mice was compared with that of $Wwox^{+/+}$ mice [Aqeilan et al., 2007b]. Fifteen weeks after administration of NMBA, almost all $Wwox^{+/-}$ mice had developed forestomach tumors, which included adenomas and invasive carcinomas. By comparison, 29% of $Wwox^{+/+}$ mice developed tumors. Intriguingly, forestomachs from $Wwox^{+/-}$ mice displayed moderately strong Wwox protein staining in the near-normal epithelium, but weak and diffuse staining in carcinomas in the same tissue section, suggesting that Wwox was haploinsufficient for the initiation of tumor development. These findings provide the first in vivo evidence for the tumor suppressor function of *WWOX* in forestomach/esophageal carcinogenesis.

Another support for the tumor suppressor function of Wwox comes from the work of the Marcelo Aldaz' laboratory. Ludes-Meyers et al. generated a hypomorphic mouse strain that had no detectable Wwox protein in most of the tissues examined. A low level could be detected in some tissues [Ludes-Meyers et al., 2007]. Wwox hypomorphic mice are viable, though, they have a significantly shorter lifespan when compared to control wild-type mice. Importantly, female hypomorphs have a higher incidence of spontaneous B-cell lymphomas, consistent with a tumor suppressor function of Wwox.

In summary, evidence demonstrates that alterations of Wwox expression in mouse models contribute to tumor development indicating that loss of *WWOX* in human cancer may be an early event in transformed cells that provides growth advantages. These data support a *bona fide* tumor suppressor function of *WWOX*.

WWOX MOLECULAR PATHWAYS

Wwox contains two N-terminal WW domains and SDR domain (Fig. 1) [Bednarek et al., 2000]. The majority of pathways in which Wwox is implicated reveals that it acts predominantly through its first WW domain, which binds the proline–tyrosine rich motifs-PP*x*Y (where P is proline, Y is a tyrosine and *x* is any amino acid) of a partner. WW domains are small protein modules ranging from 38–40 amino acids containing two conserved tryptophan (W) residues that are spaced apart by 22 amino acids. Based on the recognition of proline-rich ligands, the WW domains can be resolved into four groups [reviewed in Sudol et al., 2005]. WW domains have been identified in a wide variety of signaling proteins and function as adapter proteins, transcriptional co-activators and as ubiquitin ligases. For example, the Yes-associated proteins (Yap) functions as a transcriptional co-activator of several target genes such as p73 [Strano et al., 2001] and ErbB4 [Komuro et al., 2003], while the E3 ligase Itch regulates the stability of transcription factors such as c-Jun [Gao et al., 2004], p73 [Rossi et al., 2005], and p63 [Rossi et al., 2006].

Several partners have been reported for the Wwox protein (Table I). Initially, AxCell Biosciences has developed a biochemical approach to map WW domain peptide–protein interactions and determined that WW domains of Wwox associate with PP*x*Y containing peptides [Hu et al., 2004]. Our in vivo validation studies confirmed these preliminary results and demonstrated that the first WW domain of Wwox belongs to Group I WW domains [Aqeilan et al., 2004c]. This finding was subsequently followed by a report showing that Wwox interacts preferably with PP*x*Y containing proteins [Ludes-Meyers et al., 2004]. Using the Wwox WW domains as a probe, Ludes-Meyers et al., screened high-density protein arrays and identified five candidate-binding partners including WW binding protein 1 (WBP-1), small membrane protein of the lysosome/late endosome (SIMPLE), NF-κB activating protein, and COTE1. The common feature of these partners is that they contain the PPxY motif; though the functional relevance of these associations is still unknown.

The first partner of the WW domain of Wwox to be identified was the p53 homologue, p73 [Aqeilan et al., 2004c]. Wwox, via its first WW domain, associates with the C-terminal PP*x*Y motif of p73. We found that Wwox binds p73 mainly in the cytoplasm thus suppressing p73-transactivation function. This sequestration, however, enhances Wwox pro-apoptotic activity; Soas2 cells

TABLE I. Wwox Partners

Wwox interacting partner	Function	Refs.
p73	Pro-apoptotic transcription factor	Aqeilan et al. [2004c]
SIMPLE	Small membrane protein of the lysosome/late endosome. Mutation of the SIMPLE gene is the molecular basis of Charcot–Marie–Tooth disease type 1C (CMT1C)	Ludes-Meyers et al. [2004]
Ap2α and γ	Transcription factors involved in mammary cell proliferation and development, frequently overexpressed in breast tumors	Aqeilan et al. [2004b]
ErbB4 intracellular domain (ICD)	Receptor tyrosine kinase. Ligand binding induces a variety of cellular responses including mitogenesis and differentiation	Aqeilan et al. [2005]
Ack1	Cdc42-regulated kinase. Its overexpression is associated with tumorigenesis	Mahajan et al. [2005]
c-Jun	Transcription factor that together with c-Fos, forms AP1. Regulates proliferation, differentiation, and apoptosis in multiple cell types	Gaudio et al. [2006]
Ezrin	Membrane cytoskeletal cross-linker that participates in cell adhesion, motility, and cell survival	Jin et al. [2006]
Runx2	Principal transcriptional regulator of osteoblast differentiation and skeletal morphogenesis	Aqeilan et al. [2008]
Dvl-2	Involved in non-canonical Wnt signaling pathway, stabilizes β-catenin eliciting proliferation and cell growth	Bouteille et al. [2009]
p53, Mdm2 non-PPxY dependent	Transcription factor involved in DNA repair, growth arrest, and apoptosis	Chang et al. [2007]
Jnk (c-jun N-terminal kinase) non-PPxY dependent	Member of MAP kinases, elicits pro- and anti-apoptotic functions	Chang et al. [2003]
Tau non-PPxY dependent	Tau gene mutations have been associated with several neurodegenerative disorders such as Alzheimer's disease	Sze et al. [2004]

co-expressing Wwox and p73β have an increased percentage of sub-G1 population as compared with Wwox or p73β alone. A point mutation in the first WW domain of Wwox, namely replacement of tyrosine (Y) 33 by arginine (R) (WwoxY33R), abrogates these functions, suggesting a specific association between Wwox and p73. Importantly, this tyrosine is necessary, though not essential, for this association. Our data also reveal that Y33 is a target for phosphorylation by the Src kinase family; Src-mediating phosphorylation of Wwox enhances its interaction with p73 [Aqeilan et al., 2004c]. The significance of Wwox-p73 location in the cytoplasm remains to be elucidated. Recently, a caspase-cleaved p73 fragment was demonstrated to localize to the mitochondria, and enhances TRAIL-induced apoptosis [Sayan et al., 2008]. It is thus possible that following association with Wwox, p73 is cleaved in the cytoplasm and enhances transcriptional-independent apoptosis.

Subsequent biochemical studies in our laboratory have identified other Wwox partners including Ap2γ and α, intracellular domain (ICD) of ErbB4 and c-Jun [Aqeilan et al., 2004b, 2005; Gaudio et al., 2006]. Wwox regulates transactivation ability of these transcription factors by sequestering them in the cytoplasm [Aqeilan and Croce, 2007]. Again, the first WW domain of Wwox is the main interacting domain with these partners. The functional and clinical relevance of these interactions has also been addressed. In collaboration with the research group of Klaus Elenius, we demonstrate that Wwox-ErbB4 association on the cell membrane or in the cytoplasm correlates with better prognosis in breast cancer patients [Aqeilan et al., 2007a]. Additionally, we documented that Wwox protects full-length ErbB4 from degradation and contributes to its stabilization, which also correlates with improved survival in breast cancer patients. Therefore, we speculate that in tumors when Wwox is absent, ICD of ErbB4 freely translocates into the nucleus and mediates survival signals; a situation that is associated with poor prognosis in breast cancer patients [Junttila et al., 2005]. In another scenario, Wwox and Ap2γ expression levels correlate with tamoxifen resistance in breast tumors suggesting a functional association between the two proteins [Guler et al., 2007].

The scope of Wwox partners also includes proteins associated with cytoskeleton remodeling [Jin et al., 2006]. Wwox associates with PKA-mediated phosphorylated Ezrin, a cytoskeleton membrane protein. Wwox, via its first WW domain, binds to PP*x*Y of Ezrin, hence regulating the apical localization of Wwox in parietal cells [Jin et al., 2006]. Disruption of Ezrin–Wwox interaction blocks the remodeling of the apical membrane cytoskeleton associated with the translocation and insertion of H,K-ATPase into the apical membrane, suggesting a role for Wwox in cytoskeleton biology. Whether Wwox tumor suppressor function involves its ability to modulate cell surface structure, adhesion, migration, or organization remains to be investigated. Interestingly, all the above-mentioned partners associate with the first WW domain of Wwox. So far, no Wwox partners appear to bind through the second WW domain of Wwox. Although the second WW domain of Wwox contains a tyrosine (Y) in the place of the second conserved tryptophan (W), the signature residue directly involved in ligand binding, the specificity of the "WY domain" toward PP*x*Y core was not supposed to change [Hu et al., 2004]. Future characterization of the "WY domain" partners will help elucidate its biological function.

Wwox also partners with the Wnt/β-catenin pathway member, Disheveled (Dvl) [Bouteille et al., 2009]. Recently, Bouteille et al., demonstrate a physical and functional association between Dvl2 and Wwox. Interestingly, Dvl proteins contain the PP*x*Y motif, however, it is not obvious whether WW domain-PP*x*Y is responsible for this association. Nevertheless, Wwox functions as an inhibitor of the Wnt/β-catenin pathway through sequestration of Dvl proteins. In vivo validation of this interaction would clarify its physiological relevance.

Studies from the Chang laboratory demonstrate several Wwox-interacting partners independent of the WW domain and PPxY motifs. For example, hyaluronidase enhanced-TNF cytotoxicity is

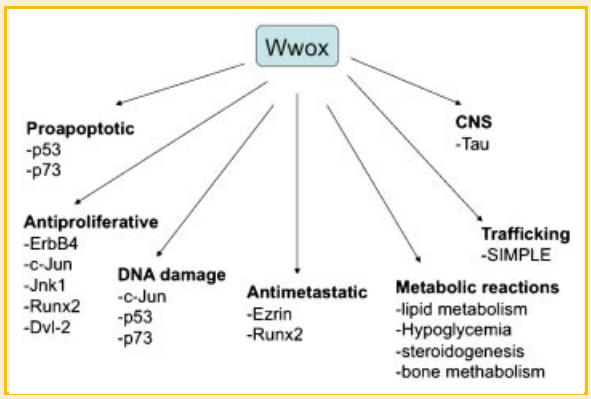

Fig. 3. Wwox Signaling pathways. Wwox is involved in multiple functions through its ability to interact with several partners of different pathways. Wwox, via its first WW domain, is able to associate with a spectrum of PP*x*Y-containing proteins and modulate their functions. For example, Wwox enhances p53 and p73 pro-apoptotic activities and decreases the transactivation activity of transcription factors involved in cell proliferation including ErbB4, c-Jun, and Runx2. Wwox is able to affect the Wnt/β-catenin pathway by retaining Dvl-2 in the cytoplasm. Because of its partners, Wwox might have also a role in DNA damage and in cell trafficking. *Wwox*-knockout mice display a wide range of metabolic defects, including hypoglycemia, hypocholesteremia, as well as morphologic steroidogenesis and bone defects, suggesting Wwox is a key regulator in different metabolic processes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

mediated through induction of Wwox, accompanied by an association with the tumor suppressor p53 [Chang et al., 2001]. Although p53 does not have a proline-rich motif, it is possible that the p53bp2, which contains a PP*x*Y motif and associates with Yap [Espanel and Sudol, 2001], mediates the p53-Wwox observed association. Wwox also associates with Jnk1 [Chang et al., 2003], Tau [Sze et al., 2004], and Mdm2 [Chang et al., 2005]. Additional investigation on the functional and biological relevance of such interactions is required in order to shed light on the Wwox signaling pathway. Nevertheless, the nature of the various interacting partners with which Wwox associates suggest that Wwox plays a central role in various signal transduction pathways (Fig. 3).

Of note, WW domain-containing proteins may have common partners, with opposite impact on their functions. For instance, Yap stabilizes p73 and enhances its DNA damage-mediated pro-apoptotic function [Strano et al., 2001; Basu et al., 2003; Levy et al., 2007] whereas the Itch E3 ligase ubiqutylates p73 and enhances its degradation [Rossi et al., 2005]. Therefore, Wwox might compete with other WW domain-containing proteins such as Yap and Itch for binding common target proteins, hence determining their functional outcome. This scenario has been tested on the ErbB4 tyrosine receptor kinase. In one study, we demonstrated that similar to Yap, Wwox associates with the ICD of ErbB4; whereas Yap co-activates ICD transactivation function [Komuro et al., 2003], the presence of Wwox, through competing for interaction with ICD, suppresses this co-activation [Aqeilan et al., 2005]. In another study we showed that Itch ubiqutylates ErbB4-CYT-1 isoform and promotes its degradation [Sundvall et al., 2008]. Therefore, it is possible that the different WW domain-containing proteins regulate the expression, localization, and function of common partners depending on their affinity of interaction and expression profile in different physiological and pathological contexts.

PHENOTYPIC ANALYSIS OF THE *WWOX*-KNOCKOUT MICE

Wwox-deficient mice on the *C57bl/6-SVJ129* genetic background are born with no obvious malformations [Aqeilan et al., 2007c, 2008]. However, these mice display a metabolic disorder characterized by hypoglycemia and hypocalcaemia at the age of 2 weeks and die by age of 3–4 weeks [Aqeilan et al., 2008]. The mice display growth retardation and have an impaired ratio of organ/body mass in several tissues including spleen, thymus, and brain [Aqeilan et al., 2008]. Although *Wwox*-deficient mice are runted, they do not exhibit any abnormal behavior or impaired motor skills. At a later stage, prior to their death, *Wwox*-knockout mice became lethargic and show signs of wasting; most likely due to sever metabolic disorder indicated by the abnormal levels of electrolytes and other serum chemistries. For example, *Wwox*-deficient mice have impaired serum levels of lipids, including hypocholesteremia and hypotriglyceremia, compared with matched age and sex control littermates. Intriguingly, Lee et al. [2008] identified a single nucleotide polymorphism (SNP) in the large intron eight of the *WWOX* gene that is associated with low HDL-C levels in dyslipidemic families suggesting a role of Wwox in HDL metabolism. The post-natal death of *Wwox*-knockout mice precludes investigating the physiological role of Wwox in vivo in adults. Interestingly, low levels of Wwox expression, as described in Wwox hypomorphs, rescued mice lethality and allowed monitoring adult mice for tumor incidence. A recent finding also suggests that a truncated form of *Wwox* in rats is associated with epilepsy, providing evidence for Wwox function in the central nervous system [Suzuki et al., 2009]. The complexity of the *Wwox* mutant mice phenotype supports multiple and unique roles for Wwox in vivo and suggest that other members of the WW domain- and SDR-containing proteins cannot compensate for these phenotypes.

The increased incidence of tumor formation reported in *Wwox*-knockout mice confirms Wwox oncosuppressor activity. An example is provided by the fact that mice lacking Wwox display an increase incidence of osteosarcomas [Aqeilan et al., 2007c]. Osteosarcoma originates from proliferation of undifferentiated osteoblasts. Interestingly, we observed an impaired differentiation in osteoblasts isolated from *Wwox* null mice, suggesting that osteosarcoma formation in *Wwox*-knockout mice could be related to a differentiation defect in the osteoblast compartment. In fact, Wwox seems to be essential in regulating proliferation and maturation of osteoprogenitor cells during bone formation [Aqeilan et al., 2008]. We observed an increase of Runx2 levels in *Wwox*-deficient mice both in clavaria and femur bones. This increased level might allow initiation of osteoblastogenesis, albeit osteoblasts are not able to produce sufficient bone matrix to compensate the calcium metabolic defects, thus, causing bone resorption and leading to loss of bone matrix. Therefore, since Wwox seems to have

a central role in osteoblast differentiation, its loss might promote osteosarcoma formation.

Biochemically, we have demonstrated a physical interaction between Wwox and Runx2 using co-immunoprecipitation assays [Aqeilan et al., 2008]. This association suppresses Runx2 transactivation function. Since *RUNX2* autoregulates its expression [Stein et al., 2004], we speculate that absence of Wwox stimulates Runx2 transactivation function and hence upregulates its expression level. Of note, Runx2 is a target of other WW domain-containing proteins including co-activators and ubiquitin ligases. Therefore, in absence of Wwox, the balance between the different WW domain adaptor proteins and Runx2 may determine the functional outcome of Runx2. Interestingly, Wwox interacts with Runx2 in the nucleus, when the transcription factor is already bound to chromatin, and inhibits its transcriptional activity. It is worth mentioning that Wwox contains a nuclear localization sequence (NLS) [Chang et al., 2007]. However, its predominant localization is observed in the cytoplasm [Aqeilan and Croce, 2007]. Since Runx2 levels are significantly upregulated in osteosarcoma [Papachristou and Papavassiliou, 2007], we speculate that Wwox may, at least in part, be responsible for this altered expression. Further investigation should uncover the functional role of Wwox-Runx2 in osteosarcoma.

The generation of the *Wwox*-deficient mice also shed light on the possible functions of Wwox in steroid metabolism [Aqeilan et al., 2009]. Analysis of the Wwox expression pattern in mouse tissue reveals that it has predominant expression in hormonally regulated tissues such as testis, ovary, prostate, and mammary epithelial cells [Nunez et al., 2006; Aqeilan et al., 2007c]; thus, we postulated a possible physiological role of Wwox in steroid metabolism. Indeed, our data demonstrate that *Wwox*-knockout mice display some morphological defects in the male and female reproductive systems; hypogonadism and impaired levels of steroidogenic enzymes have been observed [Aqeilan et al., 2009]. Moreover, both mRNA levels of the follicle-stimulating hormone (*Fsh*) and luteinizing hormone (*Lh*) are downregulated in the pituitary gland of *Wwox*-knockout mice. Similarly, testes from Wwox hypomorphic males have a high numbers of atrophic seminiferous tubules and reduced fertility when compared with their wild-type counterparts [Ludes-Meyers et al., 2007]. Since Wwox contains an SDR domain, it is speculated that this domain might play a critical role in steroid metabolism. Further delineation of Wwox domains function is required to address this matter.

CONCLUSIONS

The *WWOX* gene encodes a tumor suppressor that is altered in several types of cancer. Inactivation of *WWOX* in cancer cells could be due to genomic, epigenetic, and post-translational modifications. In vivo evidence, using *Wwox* mutant mice, demonstrate a *bona fide* tumor suppressor function of *WWOX*. The mechanism of action of Wwox involves apoptosis and, in a recent report, modulation of the interaction between tumor cells and the extracellular matrix [Gourley et al., 2009]. Data from several laboratories suggest that Wwox, via its WW domains, partners with PP*x*Y-containing proteins and modulates their functions. PP*x*Y-independent interactions have also been reported. Moreover, Wwox can regulate gene expression by competing with other WW domain-containing proteins, such as co-activators and ubiquitin ligases, for binding with its targets, thus, affecting their transactivation and degradation rate. The nature of the various interacting partners with which Wwox can physically associate suggests that Wwox plays a central role in different signal transduction pathways. *Wwox*-deficient mice display a very complex profile including post-natal lethality, defects in growth and impaired function of steroidogenesis and bone metabolism. To better define Wwox signaling pathway(s), it will be useful to identify all the possible PP*x*Y-containing proteins that Wwox binds. Furthermore, characterization of the biochemical function of the SDR domain would help in better understanding the role of Wwox in metabolic processes. In summary, further investigation of both domains should shed light on the role of Wwox in biology and pathology.

ACKNOWLEDGMENTS

We would like to thank Prof. Robert Stern for comments and critical reading of the manuscript. This work was supported by EU-FP7 Marie-Curie Re-integration Grant, Israel Cancer Research Fund (ICRF) and Israel Science Foundation (ISF) to R.I.A.

REFERENCES

Aqeilan RI, Croce CM. 2007. WWOX in biological control and tumorigenesis. J Cell Physiol 212(2):307–310.

Aqeilan RI, Kuroki T, Pekarsky Y, Albagha O, Trapasso F, Baffa R, Huebner K, Edmonds P, Croce CM. 2004a. Loss of WWOX expression in gastric carcinoma. Clin Cancer Res 10(9):3053–3058.

Aqeilan RI, Palamarchuk A, Weigel RJ, Herrero JJ, Pekarsky Y, Croce CM. 2004b. Physical and functional interactions between the Wwox tumor suppressor protein and the AP-2gamma transcription factor. Cancer Res 64(22):8256–8261.

Aqeilan RI, Pekarsky Y, Herrero JJ, Palamarchuk A, Letofsky J, Druck T, Trapasso F, Han SY, Melino G, Huebner K, Croce CM. 2004c. Functional association between Wwox tumor suppressor protein and p73, a p53 homolog. Proc Natl Acad Sci USA 101(13):4401–4406.

Aqeilan RI, Donati V, Palamarchuk A, Trapasso F, Kaou M, Pekarsky Y, Sudol M, Croce CM. 2005. WW domain-containing proteins, WWOX and YAP, compete for interaction with ErbB-4 and modulate its transcriptional function. Cancer Res 65(15):6764–6772.

Aqeilan RI, Donati V, Gaudio E, Nicoloso MS, Sundvall M, Korhonen A, Lundin J, Isola J, Sudol M, Joensuu H, Croce CM, Elenius K. 2007a. Association of Wwox with ErbB4 in breast cancer. Cancer Res 67(19): 9330–9336.

Aqeilan RI, Hagan JP, Aqeilan HA, Pichiorri F, Fong LY, Croce CM. 2007b. Inactivation of the Wwox gene accelerates forestomach tumor progression in vivo. Cancer Res 67(12):5606–5610.

Aqeilan RI, Trapasso F, Hussain S, Costinean S, Marshall D, Pekarsky Y, Hagan JP, Zanesi N, Kaou M, Stein GS, Lian JB, Croce CM. 2007c. Targeted deletion of Wwox reveals a tumor suppressor function. Proc Natl Acad Sci USA 104(10):3949–3954.

Aqeilan RI, Hassan MQ, de Bruin A, Hagan JP, Volinia S, Palumbo T, Hussain S, Lee SH, Gaur T, Stein GS, Lian JB, Croce CM. 2008. The WWOX tumor suppressor is essential for post-natal survival and normal bone metabolism. J Biol Chem 283(31):21629–21639.

Aqeilan RI, Hagan JP, de Bruin A, Rawahneh M, Salah Z, Gaudio E, Siddiqui H, Volinia S, Alder H, Lian JB, Stein GS, Croce CM. 2009. Targeted ablation of the WW domain-containing oxidoreductase tumor suppressor leads to impaired steroidogenesis. Endocrinology 150(3):1530–1535.

Bartkova J, Horejsi Z, Koed K, Kramer A, Tort F, Zieger K, Guldberg P, Sehested M, Nesland JM, Lukas C, Orntoft T, Lukas J, Bartek J. 2005. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 434(7035):864–870.

Basu S, Totty NF, Irwin MS, Sudol M, Downward J. 2003. Akt phosphorylates the Yes-associated protein, YAP, to induce interaction with 14-3-3 and attenuation of p73-mediated apoptosis. Mol Cell 11(1):11–23.

Bednarek AK, Laflin KJ, Daniel RL, Liao Q, Hawkins KA, Aldaz CM. 2000. WWOX, a novel WW domain-containing protein mapping to human chromosome 16q23. 3-24. 1, a region frequently affected in breast cancer. Cancer Res 60(8):2140–2145.

Bednarek AK, Keck-Waggoner CL, Daniel RL, Laflin KJ, Bergsagel PL, Kiguchi K, Brenner AJ, Aldaz CM. 2001. WWOX, the FRA16D gene, behaves as a suppressor of tumor growth. Cancer Res 61(22):8068–8073.

Bouteille N, Driouch K, Hage PE, Sin S, Formstecher E, Camonis J, Lidereau R, Lallemand F. 2009. Inhibition of the Wnt/beta-catenin pathway by the WWOX tumor suppressor protein. Oncogene 28(28):2569–2580.

Cantor JP, Iliopoulos D, Rao AS, Druck T, Semba S, Han SY, McCorkell KA, Lakshman TV, Collins JE, Wachsberger P, Friedberg JS, Huebner K. 2007. Epigenetic modulation of endogenous tumor suppressor expression in lung cancer xenografts suppresses tumorigenicity. Int J Cancer 120(1):24–31.

Chang NS, Pratt N, Heath J, Schultz L, Sleve D, Carey GB, Zevotek N. 2001. Hyaluronidase induction of a WW domain-containing oxidoreductase that enhances tumor necrosis factor cytotoxicity. J Biol Chem 276(5): 3361–3370.

Chang NS, Doherty J, Ensign A. 2003. JNK1 physically interacts with WW domain-containing oxidoreductase (WOX1) and inhibits WOX1-mediated apoptosis. J Biol Chem 278(11):9195–9202.

Chang NS, Doherty J, Ensign A, Schultz L, Hsu LJ, Hong Q. 2005. WOX1 is essential for tumor necrosis factor-, UV light-, staurosporine-, and p53-mediated cell death, and its tyrosine 33-phosphorylated form binds and stabilizes serine 46-phosphorylated p53. J Biol Chem 280(52):43100–43108.

Chang NS, Hsu LJ, Lin YS, Lai FJ, Sheu HM. 2007. WW domain-containing oxidoreductase: A candidate tumor suppressor. Trends Mol Med 13(1):12–22.

Chen T, Sahin A, Aldaz CM. 1996. Deletion map of chromosome 16q in ductal carcinoma in situ of the breast: Refining a putative tumor suppressor gene region. Cancer Res 56(24):5605–5609.

Chesi M, Bergsagel PL, Shonukan OO, Martelli ML, Brents LA, Chen T, Schrock E, Ried T, Kuehl WM. 1998. Frequent dysregulation of the c-maf proto-oncogene at 16q23 by translocation to an Ig locus in multiple myeloma. Blood 91(12):4457–4463.

Donati V, Fontanini G, Dell'Omodarme M, Prati MC, Nuti S, Lucchi M, Mussi A, Fabbri M, Basolo F, Croce CM, Aqeilan RI. 2007. WWOX expression in different histological types and subtypes of non-small cell lung cancer. Clin Cancer Res 13(3):884–891.

Driouch K, Prydz H, Monese R, Johansen H, Lidereau R, Frengen E. 2002. Alternative transcripts of the candidate tumor suppressor gene, WWOX, are expressed at high levels in human breast tumors. Oncogene 21(12):1832–1840.

Espanel X, Sudol M. 2001. Yes-associated protein and p53-binding protein-2 interact through their WW and SH3 domains. J Biol Chem 276(17):14514–14523.

Fabbri M, Iliopoulos D, Trapasso F, Aqeilan RI, Cimmino A, Zanesi N, Yendamuri S, Han SY, Amadori D, Huebner K, Croce CM. 2005. WWOX gene restoration prevents lung cancer growth in vitro and in vivo. Proc Natl Acad Sci USA 102(43):15611–15616.

Finnis M, Dayan S, Hobson L, Chenevix-Trench G, Friend K, Ried K, Venter D, Woollatt E, Baker E, Richards RI. 2005. Common chromosomal fragile site FRA16D mutation in cancer cells. Hum Mol Genet 14(10):1341–1349.

Gao M, Labuda T, Xia Y, Gallagher E, Fang D, Liu YC, Karin M. 2004. Jun turnover is controlled through JNK-dependent phosphorylation of the E3 ligase Itch. Science 306(5694):271–275.

Gaudio E, Palamarchuk A, Palumbo T, Trapasso F, Pekarsky Y, Croce CM, Aqeilan RI. 2006. Physical association with WWOX suppresses c-Jun transcriptional activity. Cancer Res 66(24):11585–11589.

Glover TW. 1998. Instability at chromosomal fragile sites. Recent Results Cancer Res 154:185–199.

Glover TW. 2006. Common fragile sites. Cancer Lett 232(1):4–12.

Gorgoulis VG, Vassiliou LV, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T, Venere M, Ditullio RA, Jr., Kastrinakis NG, Levy B, Kletsas D, Yoneta A, Herlyn M, Kittas C, Halazonetis TD. 2005. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 434(7035):907–913.

Gourley C, Paige AJ, Taylor KJ, Ward C, Kuske B, Zhang J, Sun M, Janczar S, Harrison DJ, Muir M, Smyth JF, Gabra H. 2009. WWOX gene expression abolishes ovarian cancer tumorigenicity in vivo and decreases attachment to fibronectin via integrin alpha3. Cancer Res 69(11):4835–4842.

Guler G, Uner A, Guler N, Han SY, Iliopoulos D, McCue P, Huebner K. 2005. Concordant loss of fragile gene expression early in breast cancer development. Pathol Int 55(8):471–478.

Guler G, Iliopoulos D, Guler N, Himmetoglu C, Hayran M, Huebner K. 2007. Wwox and Ap2{gamma} expression levels predict tamoxifen response. Clin Cancer Res 13(20):6115–6121.

Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. Cell 100(1):57–70.

Hu H, Columbus J, Zhang Y, Wu D, Lian L, Yang S, Goodwin J, Luczak C, Carter M, Chen L, James M, Davis R, Sudol M, Rodwell J, Herrero JJ. 2004. A map of WW domain family interactions. Proteomics 4(3):643–655.

Huebner K, Croce CM. 2003. Cancer and the FRA3B/FHIT fragile locus: It's a HIT. Br J Cancer 88(10):1501–1506.

Iliopoulos D, Guler G, Han SY, Johnston D, Druck T, McCorkell KA, Palazzo J, McCue PA, Baffa R, Huebner K. 2005. Fragile genes as biomarkers: Epigenetic control of WWOX and FHIT in lung, breast and bladder cancer. Oncogene 24(9):1625–1633.

Iliopoulos D, Fabbri M, Druck T, Qin HR, Han SY, Huebner K. 2007. Inhibition of breast cancer cell growth in vitro and in vivo: Effect of restoration of Wwox expression. Clin Cancer Res 13(1):268–274.

Jin C, Ge L, Ding X, Chen Y, Zhu H, Ward T, Wu F, Cao X, Wang Q, Yao X. 2006. PKA-mediated protein phosphorylation regulates ezrin-WWOX interaction. Biochem Biophys Res Commun 341(3):784–791.

Junttila TT, Sundvall M, Lundin M, Lundin J, Tanner M, Harkonen P, Joensuu H, Isola J, Elenius K. 2005. Cleavable ErbB4 isoform in estrogen receptor-regulated growth of breast cancer cells. Cancer Res 65(4):1384–1393.

Komuro A, Nagai M, Navin NE, Sudol M. 2003. WW domain-containing protein YAP associates with ErbB-4 and acts as a co-transcriptional activator for the carboxyl-terminal fragment of ErbB-4 that translocates to the nucleus. J Biol Chem 278(35):33334–33341.

Krummel KA, Denison SR, Calhoun E, Phillips LA, Smith DI. 2002. The common fragile site FRA16D and its associated gene WWOX are highly conserved in the mouse at Fra8E1. Genes Chromosomes Cancer 34(2):154–167.

Kuroki T, Trapasso F, Shiraishi T, Alder H, Mimori K, Mori M, Croce CM. 2002. Genetic alterations of the tumor suppressor gene WWOX in esophageal squamous cell carcinoma. Cancer Res 62(8):2258–2260.

Kuroki T, Yendamuri S, Trapasso F, Matsuyama A, Aqeilan RI, Alder H, Rattan S, Cesari R, Nolli ML, Williams NN, Mori M, Kanematsu T, Croce CM. 2004. The tumor suppressor gene WWOX at FRA16D is involved in pancreatic carcinogenesis. Clin Cancer Res 10(7):2459–2465.

Latil A, Cussenot O, Fournier G, Driouch K, Lidereau R. 1997. Loss of heterozygosity at chromosome 16q in prostate adenocarcinoma: Identification of three independent regions. Cancer Res 57(6):1058–1062.

Lee JC, Weissglas-Volkov D, Kyttala M, Dastani Z, Cantor RM, Sobel EM, Plaisier CL, Engert JC, van Greevenbroek MM, Kane JP, Malloy MJ, Pullinger CR, Huertas-Vazquez A, Aguilar-Salinas CA, Tusie-Luna T, de Bruin TW, Aouizerat BE, van der Kallen CC, Croce CM, Aqeilan RI, Marcil M, Viikari JS, Lehtimaki T, Raitakari OT, Kuusisto J, Laakso M, Taskinen MR, Genest J, Pajukanta P. 2008. WW-domain-containing oxidoreductase is associated with low plasma HDL-C levels. Am J Hum Genet 83(2):180–192.

Levy D, Adamovich Y, Reuven N, Shaul Y. 2007. The Yes-associated protein 1 stabilizes p73 by preventing Itch-mediated ubiquitination of p73. Cell Death Differ 14(4):743–751.

Ludes-Meyers JH, Kil H, Bednarek AK, Drake J, Bedford MT, Aldaz CM. 2004. WWOX binds the specific proline-rich ligand PPXY: Identification of candidate interacting proteins. Oncogene 23(29):5049–5055.

Ludes-Meyers JH, Kil H, Nunez MI, Conti CJ, Parker-Thornburg J, Bedford MT, Aldaz CM. 2007. WWOX hypomorphic mice display a higher incidence of B-cell lymphomas and develop testicular atrophy. Genes Chromosomes Cancer 46(12):1129–1136.

Mahajan NP, Whang YE, Mohler JL, Earp HS. 2005. Activated tyrosine kinase Ack1 promotes prostate tumorigenesis: Role of Ack1 in polyubiquitination of tumor suppressor Wwox. Cancer Res 65(22):10514–10523.

Mangelsdorf M, Ried K, Woollatt E, Dayan S, Eyre H, Finnis M, Hobson L, Nancarrow J, Venter D, Baker E, Richards RI. 2000. Chromosomal fragile site FRA16D and DNA instability in cancer. Cancer Res 60(6):1683–1689.

Nakayama S, Semba S, Maeda N, Aqeilan RI, Huebner K, Yokozaki H. 2008. Role of the WWOX gene, encompassing fragile region FRA16D, in suppression of pancreatic carcinoma cells. Cancer Sci 99(7):1370–1376.

Nunez MI, Ludes-Meyers J, Aldaz CM. 2006. WWOX protein expression in normal human tissues. J Mol Histol 37(3–4):115–125.

Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM, Huebner K. 1996. The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancers. Cell 84(4): 587–597.

O'Keefe LV, Richards RI. 2006. Common chromosomal fragile sites and cancer: Focus on FRA16D. Cancer Lett 232(1):37–47.

Paige AJ, Taylor KJ, Stewart A, Sgouros JG, Gabra H, Sellar GC, Smyth JF, Porteous DJ, Watson JE. 2000. A 700-kb physical map of a region of 16q23.2 homozygously deleted in multiple cancers and spanning the common fragile site FRA16D. Cancer Res 60(6):1690–1697.

Paige AJ, Taylor KJ, Taylor C, Hillier SG, Farrington S, Scott D, Porteous DJ, Smyth JF, Gabra H, Watson JE. 2001. WWOX: A candidate tumor suppressor gene involved in multiple tumor types. Proc Natl Acad Sci USA 98(20): 11417–11422.

Palakodeti A, Han Y, Jiang Y, Le Beau MM. 2004. The role of late/slow replication of the FRA16D in common fragile site induction. Genes Chromosomes Cancer 39(1):71–76.

Papachristou DJ, Papavassiliou AG. 2007. Osteosarcoma and chondrosarcoma: New signaling pathways as targets for novel therapeutic interventions. Int J Biochem Cell Biol 39(5):857–862.

Qin HR, Iliopoulos D, Semba S, Fabbri M, Druck T, Volinia S, Croce CM, Morrison CD, Klein RD, Huebner K. 2006. A role for the WWOX gene in prostate cancer. Cancer Res 66(13):6477–6481.

Ried K, Finnis M, Hobson L, Mangelsdorf M, Dayan S, Nancarrow JK, Woollatt E, Kremmidiotis G, Gardner A, Venter D, Baker E, Richards RI. 2000. Common chromosomal fragile site FRA16D sequence: Identification of the FOR gene spanning FRA16D and homozygous deletions and translocation breakpoints in cancer cells. Hum Mol Genet 9(11):1651–1663.

Rossi M, De Laurenzi V, Munarriz E, Green DR, Liu YC, Vousden KH, Cesareni G, Melino G. 2005. The ubiquitin-protein ligase Itch regulates p73 stability. EMBO J 24(4):836–848.

Rossi M, Aqeilan RI, Neale M, Candi E, Salomoni P, Knight RA, Croce CM, Melino G. 2006. The E3 ubiquitin ligase Itch controls the protein stability of p63. Proc Natl Acad Sci USA 103(34):12753–12758.

Sayan AE, Sayan BS, Gogvadze V, Dinsdale D, Nyman U, Hansen TM, Zhivotovsky B, Cohen GM, Knight RA, Melino G. 2008. P73 and caspase-cleaved p73 fragments localize to mitochondria and augment TRAIL-induced apoptosis. Oncogene 27(31):4363–4372.

Stein GS, Lian JB, van Wijnen AJ, Stein JL, Montecino M, Javed A, Zaidi SK, Young DW, Choi JY, Pockwinse SM. 2004. Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression. Oncogene 23(24):4315–4329.

Strano S, Munarriz E, Rossi M, Castagnoli L, Shaul Y, Sacchi A, Oren M, Sudol M, Cesareni G, Blandino G. 2001. Physical interaction with Yes-associated protein enhances p73 transcriptional activity. J Biol Chem 276(18):15164–15173.

Sudol M, Recinos CC, Abraczinskas J, Humbert J, Farooq A. 2005. WW or WoW: The WW domains in a union of bliss. IUBMB Life 57(12):773–778.

Sundvall M, Korhonen A, Paatero I, Gaudio E, Melino G, Croce CM, Aqeilan RI, Elenius K. 2008. Isoform-specific monoubiquitination, endocytosis, and degradation of alternatively spliced ErbB4 isoforms. Proc Natl Acad Sci USA 105(11):4162–4167.

Suzuki H, Katayama K, Takenaka M, Amakasu K, Saito K, Suzuki K. 2009. A spontaneous mutation of the Wwox gene and audiogenic seizures in rats with lethal dwarfism and epilepsy. Genes Brain Behav (in press).

Sze CI, Su M, Pugazhenthi S, Jambal P, Hsu LJ, Heath J, Schultz L, Chang NS. 2004. Down-regulation of WW domain-containing oxidoreductase induces Tau phosphorylation in vitro. A potential role in Alzheimer's disease. J Biol Chem 279(29):30498–30506.

Yendamuri S, Kuroki T, Trapasso F, Henry AC, Dumon KR, Huebner K, Williams NN, Kaiser LR, Croce CM. 2003. WW domain containing oxidoreductase gene expression is altered in non-small cell lung cancer. Cancer Res 63(4):878–881.

Yu S, Mangelsdorf M, Hewett D, Hobson L, Baker E, Eyre HJ, Lapsys N, Le Paslier D, Doggett NA, Sutherland GR, Richards RI. 1997. Human chromosomal fragile site FRA16B is an amplified AT-rich minisatellite repeat. Cell 88(3):367–374.