Minireview

Signaling from membrane receptors to tumor suppressor WW domain-containing oxidoreductase

Jean-Yun Chang[1], Ruei-Yu He[2], Hsin-Ping Lin[1], Li-Jin Hsu[3,4], Feng-Jie Lai[5], Qunying Hong[6], Shean-Jen Chen[2] and Nan-Shan Chang[1,4,7]

[1]Institute of Molecular Medicine; [2]Department of Engineering Science; [3]Department of Medical Laboratory Science and Biotechnology; [4]Center for Gene Regulation and Signal Transduction Research, National Cheng Kung University Medical College; [5]Department of Dermatology, Chi-Mei Medical Center, Tainan, Taiwan; [6]Department of Pulmonary Medicine, Zhongshan Hospital, Fudan University, Shanghai, China; [7]Department of Neuroscience and Physiology, SUNY Upstate Medical University, Syracuse, NY, USA
Corresponding authors: Li-Jin Hsu or Nan-Shan Chang. Emails: lijin.hsu@gmail.com or nschang13827@gmail.com

Abstract

The family of WW domain-containing proteins contains over 2000 members. The small WW domain module is responsible, in part, for protein/protein binding interactions and signaling. Many of these proteins are located at the membrane/cytoskeleton area, where they act as adaptors to receive signals from the cell surface. In this review, we provide molecular insights regarding recent novel findings on signaling from the cell surface toward WW domain-containing oxidoreductase, known as WWOX, FOR or WOX1. More specifically, transforming growth factor beta 1 utilizes cell surface hyaluronidase Hyal-2 (hyaluronoglucosaminidase 2) as a cognate receptor for signaling with WWOX and Smad4 to control gene transcription, growth and death. Complement C1q alone, bypassing the activation of classical pathway, signals a novel event of apoptosis by inducing microvillus formation and WWOX activation. Deficiency in these signaling events appears to favorably support cancer growth.

Keywords: signaling, TGF-β, WOX1, FOR, WWOX, hyaluronidase Hyal-2, complement C1q

Experimental Biology and Medicine 2010; **235**: 796–804. DOI: 10.1258/ebm.2010.009351

WW domain-containing proteins in signaling network

WW domain is a small, conserved structural module that is involved in protein/protein interactions.[1–4] WW domains support the assembly of multiprotein interaction networks.[3,4] The domain comprises a stretch of 35–40 amino acids with two conserved tryptophan residues and a conserved proline.[1,2] The WW domains can be categorized as four groups, and each group preferentially interacts with proteins containing particular proline motifs and/or phosphoserine- or phosphothreonine-containing motifs.[1–4] For example, group I WW domain preferentially interacts with PPxY motif and group II with PPLP.[3,4] Group II/III WW domains can bind simple polyprolines (PPPP) and PL and PR motifs.[5] There are more than 2000 WW domain family members, and many of these proteins possess one or even 2–4 WW domains. The WW domains can be arranged in tandem closely and at various distances in a single protein.[6] These domains may act in a synergistic or an independent manner.[6]

WW domain proteins exhibit a multitude of functional diversities, as reflected by their roles in highly complicated signaling networks, and involvement in physiological development and metabolic disorders.[7–12] During signaling, numerous cytosolic WW domain proteins are responsive to the challenge of extracellular cues. WW domain-containing proteins YAP (Yes-associated protein; 2 WW domains) and TAZ (transcriptional co-activator with PDZ-binding motif; 1 WW domain) are signal transducers from the cytoplasm to the nucleus for gene transcription, which is critical in embryonic development, tumorigenesis and other pathogenic processes.[7] Under the WW domain–PPxY binding rule, the WW domain of TAZ and YAP binds transcription factors such as polyomavirus enhancer binding protein 2α, activating protein 2, C/EBPα (CCAAT/enhancer-binding protein), c-Jun, Krox-20, Krox-24, myocyte enhancer factor 2B, nuclear factor (NF)-E2, Oct-4, Smad7, p53BP2, runt-related transcription factor 2 (RUNX2), ErbB4 and p73α.[8,10] Mice lacking *TAZ* gene develop multiple renal cysts, urinary concentration defects and pulmonary emphysematous changes.[10]

Similarly, in the bone morphogenetic protein signaling, the second WW domain of Smad ubiquitin regulatory factor 1 (Smurf1) interacts with the PPxY motif in Smads 1, 5 and 6.[11] Smurf1 is an E3 ubiquitin ligase that catalyzes ubiquitination of target proteins for proteasomal degradation.

Neuronal precursor cell-expressed developmentally downregulated 4 (NEDD4)-like family of E3 ubiquitin ligases, possessing two to four WW domains, participates in numerous signal pathways (e.g. transforming growth factor-beta [TGF-β], epidermal growth factor [EGF], insulin growth factor [IGF] and tumor necrosis factor [TNF]) via regulation of ubiquitin-mediated trafficking, lysosomal or proteasomal degradation and protein nuclear translocation.[12] These proteins have been implicated in their critical roles for cancer growth regulation.

Peptidylprolyl *cis/trans* isomerase, NIMA-interacting 1 (Pin1), containing only a WW domain considered as group III[4] or group IV,[5] is involved in both cancer and Alzheimer's disease.[13,14] Pin1 interacts with proteins possessing motifs including PPPP, PxPPxR, PPLP, PPRxP and PPxY.[4] Pin1 catalyzes phosphorylation-dependent isomerization of phospho-serine/threonine-proline motifs, i.e. conversion of peptidyl proline bond from *cis* to *trans*. Pin1 is upregulated in cancers but downregulated in Alzheimer's disease, and participates in immune regulation.[15] How Pin1 precisely contributes to diseases remains elusive.

Tumor suppressor WW domain-containing oxidoreductase

Cumulative studies both *in vitro* and *in vivo* show that WW domain-containing oxidoreductase (WWOX), designated as human WWOX or FOR, or murine WOX1, is a functional tumor suppressor (see refs.[16–22] for reviews). Human *WWOX* or mouse *Wwox* gene encodes a 46-kDa WWOX/FOR/WOX1 protein. In addition, several reports determined that WWOX participates in neural development, neuronal damage and neurodegeneration,[23–27] postnatal survival and normal bone metabolism,[28,29] and development of reproductive system.[30,31] In humans, *WWOX* is identified as one of the genes that are involved in osteoarthritis and their collaborative metabolic and inflammatory networks, as determined by integrative micro-RNA and proteomic approaches.[32] Low serum HDL-cholesterol contributes significantly to coronary artery disease. A recent study determined that an identified single-nucleotide polymorphism locus, rs2548861, in *WWOX* gene is associated with low plasma HDL-cholesterol levels.[33] Rats with lethal dwarfism and epilepsy develop audiogenic seizures and a spontaneous mutation of *Wwox* gene.[34] WWOX could be either proapoptotic or prosurvival in physiological and pathological settings. Whether environmental cues induce changes of WWOX functions is largely unknown.

Human *WWOX* gene is mapped to chromosome 16q23.3–24.1, or on a common fragile site FRA16D (see refs.[16–22] for reviews). This gene contains nine exons and generates at least eight alternatively spliced mRNAs. Full-length WWOX/WOX1 is composed of two N-terminal WW domains and a C-terminal short-chain alcohol dehydrogenase/reductase (ADH/SDR) domain (Figure 1). Like most

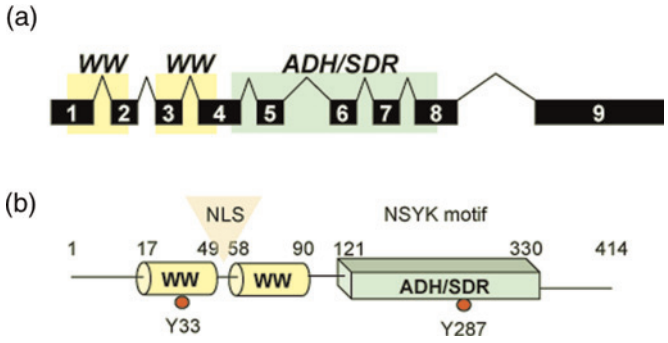

Figure 1 Schematic diagram of WWOX gene and protein. *WWOX* gene contains nine exons and encodes a 414-amino-acid WWOX, designated human WWOX or FOR, or murine WOX1 (molecular weight 46 kDa). Two N-terminal WW domains are encoded by exons 1–4, and short-chain alcohol dehydrogenase/reductase (ADH/SDR) domain by exons 4–8. There is a nuclear localization signal between the two WW domains, and an NSYK motif in the ADH/SDR domain. Two conserved tyrosine phosphorylation sites, Tyr33 and Tyr287, are shown. WWOX, WW domain-containing oxidoreductase (A color version of this figure is available in the online journal)

ADH/SDR-containing proteins, WWOX possesses an NSYK (Asn-Ser-Tyr-Lys) motif, which may bind hormone substrates. A nuclear localization signal is located between the first and second WW domains, and a mitochondria-targeting sequence is mapped in the ADH/SDR domain.[35] Localization of WWOX in cells may vary depending upon the status of cell differentiation and the types of cells tested.[17] For example, WWOX/WOX1 is frequently localized in the perinuclear area of keratinocytes at the stratum basale and spinosum of the skin epidermis. When keratinocytes start undergoing cornification at the stratum granulosum, WWOX/WOX1 is then accumulated in the nucleus.[17,36]

Numerous stress stimuli have been shown to induce re-location of WWOX/WOX1 to the nuclei. In response to TNF-α, TGF-β, staurosporine, etoposide, ultraviolet (UV) irradiation, complement C1q and sex steroid hormones estrogen and androgen, phosphorylation at Tyr33 in WWOX/WOX1 occurs in many cultured cell lines.[37–41] The phosphorylated or activated WWOX/WOX1 then translocates from the cytosol to the mitochondria and nuclei.[37–41] Src tyrosine kinase is reported to phosphorylate Tyr33.[42] For breast cancer cells (e.g. Michigan Cancer Foundation-7 [MCF7]), WWOX is refractory to re-location to the nucleus upon stimulation with sex steroid hormones.[39] A likely scenario is that WWOX is tightly bound by Ezrin,[43] where both proteins are localized at the membrane/cytoskeleton area.[40,41,43]

Compelling evidence for nuclear accumulation of WOX1 has been shown in several experimental models *in vivo*. For example, long-term exposure of rats to constant light induces accumulation of WOX1 in the mitochondria and nuclei of damaged photoreceptors in the rat retinas.[25] Intracranial injection of rats with neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) induces Parkinson's-like disease, and WOX1 is accumulated in the nuclei of damaged neurons in the striatum and cortex.[26] Also, transection of the sciatic nerve in rats causes dramatic activation or nuclear accumulation of WOX1 and transcription factors cAMP response element binding (CREB), NF-κB and many others.[27] Nuclear localization of

these proteins may ultimately lead to the loss of small dorsal root ganglion neurons postaxotomy for 2–3 months or longer.[27] We validated these *in vivo* observations regarding nuclear accumulation of WOX1 by immunohistochemistry, immunofluorescence microscopy and immunoelectron microscopy using specific homemade and commercial antibodies.[23–27]

WWOX acts more than a tumor suppressor

Over the past 10 years since the discovery of WWOX/FOR/WOX1,[35,44,45] substantial efforts from global scientists have been dedicated to the characterization of *WWOX* gene alterations in different types of cancer cells. Many comprehensive review articles have addressed the relationship between *WWOX/Wwox* gene and cancer development (see refs[16–22,46–49] for reviews). Both gene and protein expression levels of WWOX have been shown to be dramatically downregulated or lost mostly in invasive cancer cells derived from breast, prostate, esophagus, lung, pancreas, stomach, bladder, extrahepatic bile duct, cervix, thyroid and leukemia. As a tumor suppressor, expression of WWOX in cancer tissues correlates with better prognosis for patients.

In a knockout model by targeting exons 2–4 of mouse *Wwox* gene, spontaneous tumor formation in the heterozygous mice occurs at 17.2% (10/58), compared with 3.3% (2/60) in the wild type.[50] The percentages are indeed low. However, carcinogen induces cancer formation at a significantly higher incidence in heterozygous mice than in the wild type.[50] Gene-trap targeting of mouse *Wwox* gene increases the incidence of spontaneous formation of B-cell lymphomas.[30] Most recently, a report shows that conditional *Wwox* knockout mice with exon 1 ablation did not develop spontaneous tumor formation.[29] Difference in the results regarding spontaneous tumor formation is unknown and yet to be elucidated.

Wwox−/− mice with exons 2–4 ablation are significantly reduced in size, survive for less than a month and could suffer metabolic bone disorder.[28,50] These mice also have problems in the reproductive system.[31] Exon 1-ablated mice also exhibit severe metabolic defect, growth retardation, bone metabolic defect, hypocapnia (reduced CO_2 levels in the blood) and impaired hematopoiesis, leukopenia and splenic atrophy.[29] Clearly, murine *Wwox* gene plays a critical role in cell differentiation and embryonic development.

In other biological functions, we have determined that WWOX/WOX1 may play a critical role in supporting organogenesis and regulating tumorigenesis. Endogenous mouse WOX1 is significantly increased at both gene and protein levels during embryonic development.[24] Most intriguingly, protein levels of WWOX are significantly increased in the early stages of hyperplasia and cancerous progression of breast and prostate in humans.[39,49] Also, WWOX/WOX1 is upregulated during normal skin keratinocyte differentiation, as well as in the early stages of UVB-induced formation of squamous cell carcinoma in humans and mice.[36] This upregulation can be interpreted as WWOX/WOX1 has just entered the battling ground to block cancer growth. However, other likely scenarios cannot be excluded. Overall, in addition to its role in tumor suppression, WWOX/WOX1 is central to normal cell survival, differentiation and organogenesis.

WWOX is a sensor of extracellular cues

Substantial evidence shows that tumor suppressor WWOX acts as a molecular sensor of extracellular cues. Detection of environmental changes is important for cell survival. Certain WW domain-containing proteins are localized in the membrane/cytoskeleton area.[40,41,43,51,52] For example, WW domain-containing E3 ubiquitin protein ligase 1, known as WWP1, targets the full-length ErbB4 for ubiquitin-mediated degradation in breast cancer.[53] WWP1 is normally associated with membranous structures in organelles. WWOX receives and integrates signals from the cell surface by undergoing Tyr33 phosphorylation and re-location to the nuclei *in vitro*[17,35–39,52,54] and *in vivo*.[25–27,36] As a result, nuclear WWOX may either enhance or inhibit transcription factors for regulating promoter activation *in vivo*.[27] Direct close contact of prostate LNCaP cells with human vascular endothelial cells results in reduction in signaling via, in part, suppression of WWOX phosphorylation at Tyr33.[52] However, in certain cells, WWOX is retained in the membrane/cytoskeleton area by associating with Ezrin[43] or membrane Hyal-2 (hyaluronoglucosaminidase 2).[41]

Many WW domain-containing proteins transmit extracellular signals via their WW domains.[1–4] This could result in either inhibition or enhancement of the signal transduction. Both the first WW domain and the ADH/SDR domain of WWOX participate in protein–protein interaction.[17] Whether both domains interact with each other is unknown. Also, whether conformational changes occur in WWOX during signal transduction remain to be established. The WW domain of WWOX belongs to the group I WW domain, which mainly recognizes the proline-rich PPxY motif.[3–6] The known PPxY-binding partners for WWOX include p73,[42] activator protein 2γ (AP-2γ),[55] ErbB4,[56,57] Ezrin,[43] small integral membrane protein of the lysosome/late endosome (SIMPLE),[58] c-Jun[27,59] and RUNX2[28] (Figure 2). Indeed, it is not surprising to find there are thousands of proteins in the protein database possessing the PPxY motif, suggesting their likely interactions with WWOX.

In transient overexpression experiments, WWOX suppresses the transcriptional function of p73, AP-2γ, ErbB4 and c-Jun by sequestering these factors in the cytoplasm of cells.[42,55–57,59] These *in vitro* findings do not appear to be true *in vivo*. Upon axotomy of the sciatic nerve in rats, accumulation of phosphorylated WOX1, Jun N-terminal kinase1 (JNK1), CREB, c-Jun, NF-κB and activating transcription factor 3 in the nuclei of injured neurons took place within hours or the first week of injury.[27] By immunoelectron microscopy, we demonstrated the complex formation of p-WOX1 (Tyr33 phosphorylated) with p-CREB and p-c-Jun in the nuclei of neurons of transected sciatic nerve in rats.[27] FRET (Förster/fluorescence resonance energy transfer) microscopy analysis also demonstrated

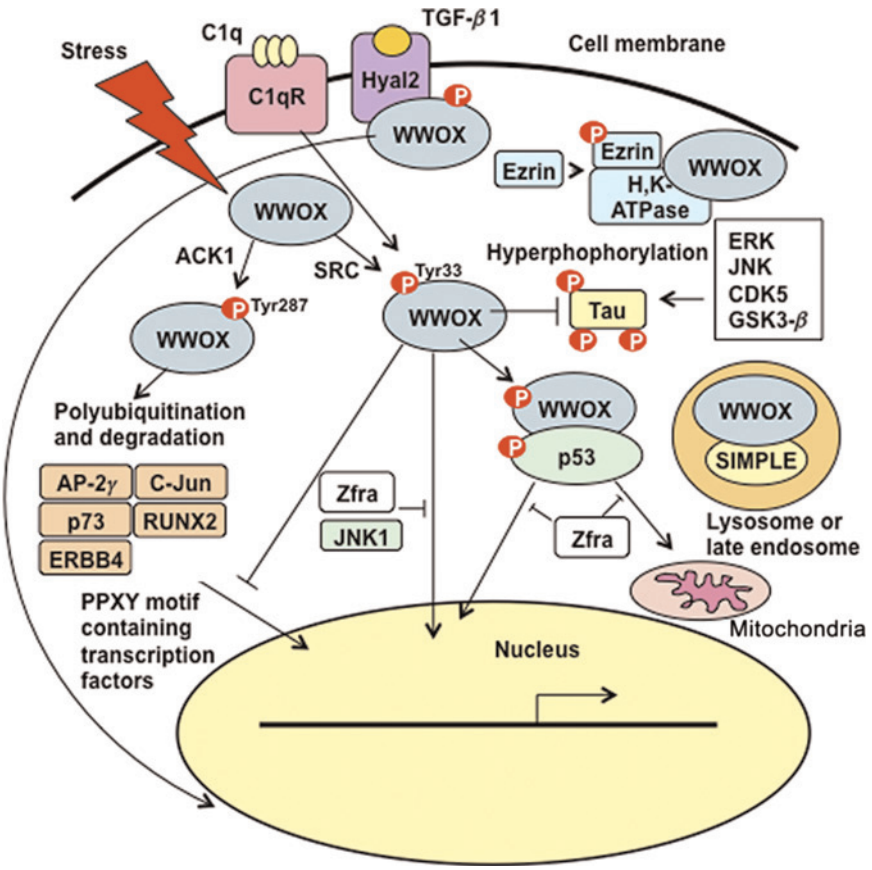

Figure 2 WWOX in signaling. The first WW domain of WWOX/WOX1 physically interacts with proteins possessing a proline-rich PPXY motif(s). These proteins include p73,[42] AP-2γ,[55] ErbB4,[53,57] Ezrin,[43] SIMPLE,[58] c-Jun,[27,59] RUNX2[28] and many others. Transiently overexpressed WWOX suppresses the transcriptional function of p73, AP-2γ, ErbB4 and c-Jun by sequestering these factors in cytoplasm of cells. Tyr33-phosphorylated WOX1/WWOX binds p53, JNK1, MDM2, Zfra, and Hyal-2 in a PPxY motif-independent manner.[35,37,38,41,60,61] JNK1 and Zfra counteract the apoptotic function of WOX1/WWOX.[37,60,61] Both Zfra and Tau interact with WOX1/WWOX via the C-terminal ADH/SDR domain.[23,60] Ack1 induces Tyr287 phosphorylation of WWOX, which allows degradation of WWOX via ubiquitin/proteasome pathway.[62] WWOX, WW domain-containing oxidoreductase; AP-2γ, activator protein 2γ; SIMPLE, small integral membrane protein of the lysosome/late endosome; RUNX2, runt-related transcription factor 2; Ack1, activated Cdc42-associated kinase 1; JNK1, Jun N-terminal kinase1; Zfra, 31-amino-acid zinc finger-like protein; ADH/SDR, C-terminal short-chain alcohol dehydrogenase/reductase; Hyal-2, hyaluronoglucosaminidase 2

that the WW domain area of WOX1 binds CREB most strongly in the nucleus.[27] WOX1 blocks the prosurvival CREB-, CRE- and AP-1-mediated promoter activation *in vitro*, but enhances promoter activation governed by c-Jun, Ets-like gene-1 and NF-κB.[27] A clear difference from *in vitro* studies is that we determined that WOX1/WWOX enhances c-Jun-regulated promoter activation,[27] whereas one report showed an inhibitory effect on the promoter activation.[59] The difference remains to be resolved.

Once phosphorylated at Tyr33 in the first WW domain, WOX1/WWOX interacts with p53,[16,17,26,35,37,38] JNK1,[26,37] murine double minute 2 (MDM2),[38] Zfra (31-amino-acid zinc finger-like protein)[60,61] and Hyal-2[41] independently of the PPxY-binding manner. Tau, a microtubule-binding protein involved in neurodegeneration, physically interacts with WOX1/WWOX via the C-terminal ADH/SDR domain.[20] In addition to the WW domain, the small size 3.5-kDa Zfra also interacts with the ADH/SDR domain.[60,61] Activated Cdc42-associated kinase 1 (Ack1), a cytoplasmic tyrosine kinase capable of binding toEGF receptor and clathrin, induces Tyr287 phosphorylation of WWOX for degradation via the ubiquitin/proteasome-dependent pathway.[62] Interestingly, the E3 ubiquitin ligase NEDD4-2, a WW domain-containing protein named neural precursor cell expressed, developmentally downregulated 4-2, suppresses active Ack1 via proteosomal degradation,[63] implying the action may increase the stability of WWOX. MDM2 physically interacts with WWOX.[38] When MDM2 is knocked down, the binding interactions between p53 and WWOX and their stability are increased,[38] suggesting that MDM2 may act as the E3 ubiquitin ligase for WWOX in degradation.

In vivo experiments have also revealed that WWOX acts as a molecular sensor during physiological and pathological events, as well as environmental stress. *WWOX* is one of the genes that are responsive to environmental challenge such as UV radiation.[64] We have determined the rapid induction of WOX1 protein expression in the epidermis of acute UVB-irradiated hairless mice.[36] Prolonged constant light exposure for two months in rats causes their retinal damage, as evidenced by accumulation of activated WOX1/WWOX in the apoptotic mitochondria and nuclei of photoreceptors of retinas.[25] Also, accumulation of activated WOX1/WWOX occurs during neurotoxin MPP^+-induced neuronal death in the cortex and striatum

of the brain in rat.[26] Collectively, environmental stress induces activation of WWOX, which links to large signaling networks and transcriptional regulation.

WW domain-containing proteins in TGF-β signal pathway

Many WW domain-containing proteins participate in the TGF-β signaling. WWP1, WW domain-containing E3 ubiquitin protein ligase 1,[53] negatively regulates TGF-β signaling by interacting with Smad7 for nuclear export.[65] In addition, WWP1 inhibits TGF-β-induced phosphorylation of Smad2/3[65] and promotes Smad4 degradation.[66] WWP1 enhances the binding of Smad7 with TGF-β type I receptor (TβRI), thus allowing ubiquitination and degradation of the receptor. Similarly, WW domain-containing NEDD4-2 is a negative regulator of TGF-β signaling.[66] NEDD4-2 is a member of the Smurf-like C2-WW-HECT-type E3 ubiquitin ligases. NEDD4-2 binds Smad7 for interacting with TβRI, which is needed for ubiquitin-dependent degradation of the receptor. Moreover, NEDD4-2 interacts with Smads 2 and 3, and causes degradation of Smad2, but not Smad3.[67]

A novel TGF-β/Hyal-2/WWOX/Smad4 signal pathway

Most recently, we demonstrated that TGF-β induces re-location of WOX1/WWOX to the nuclei in response to TGF-β1 in many types of cells, except in breast cancer cells.[41] It is generally believed that TGF-β1 binds membrane TβRII as a cognate receptor for recruiting TβRI, followed by phosphorylating Smads 2 and 3.[68,69] Upon further recruiting Smad4, the signaling complex Smad2/3/4 translocates to the nuclei for controlling gene transcription.[68,69] By utilizing TβRII-deficient HCT116 cells, we show that membrane hyaluronidase Hyal-2 is a cognate receptor for TGF-β1.[41]

TGF-β is considered as a potent inhibitor of epithelial cell growth and an architect for the extracellular matrix. TGF-β appears to play a dual role in cell growth regulation.[68–71] Alteration of TGF-β signaling such as loss of TβRII in intestinal epithelial cells promotes the invasion and malignant transformation of tumors due to increased expression of matrix metalloproteinase (MMP)-2 and MMP-9 and TGF-β1 secretion, as initiated by *Apc* mutation.[72] Metalloproteinases indeed play an important role in TGF-β-regulated metastasis.[73] Interestingly, Gr-1+ CD11b+ myeloid cells are recruited into mammary carcinomas in TβRII gene knockout mice and contribute to TGF-β-mediated metastasis.[74] TGF-β1 knockout mice exhibit defects in the hyperplasty/adenoma transition, inability to maintain epithelial tissue organization but not loss in growth control, inflammatory activity and genetic instability.[75] Together, invasive cancer cells frequently overproduce TGF-β to promote their growth and metastasis by epithelial–mesenchymal transition.[70,71] Metastatic cancer cells are frequently deficient in TβRII, which correlates with poor prognosis in patients.[76]

Dramatic upregulation of hyaluronan and hyaluronidases Hyal-1, Hyal-2 and PH-20 has been shown to be associated with cancer metastasis.[77–79] We have first demonstrated that hyaluronidases counteract the activity of TGF-β1 in growth regulation.[80–82] For example, TGF-β1 inhibits the growth of normal epithelial cells, whereas PH-20 counteracts the effect of TGF-β1.[80,81] TGF-β1 protects murine L929 fibroblasts from the cytotoxic effect of TNF,[83] and Hyal-1 and Hyal-2 reverse the TGF-β1 function.[80–82] Hyaluronidases PH-20, Hyal-1 and Hyal-2 induce the expression of WWOX/WOX1, and enhance its apoptotic function *in vitro*.[35,82] Metastatic cancer cells overly produce TGF-β, hyaluronan and hyaluronidases, and how these molecules coordinate effectively in promoting cancer growth and progression is largely unknown.

We have determined that TGF-β1 binds cell surface hyaluronidase Hyal-2 in microvilli in TβRII-deficient colon HCT116 cells.[41] TGF-β1 binds to a surface-exposed segment in the catalytic domain of Hyal-2 in the microvilli (determined by yeast two-hybrid analysis), followed by rapidly recruiting WWOX (Figure 3). The resulting WWOX/Hyal-2 complex, which further recruits Smad4, re-locates to the nuclei for enhancing Smad promoter activation (Figure 3). Most importantly, ectopic WWOX and Hyal-2, when in combination, dramatically enhances the Smad promoter activation (8- to 9-fold increases), which subsequently leads to cell death (>95% of promoter-activated cells),[41] raising the possibility that Hyal-2 and WWOX bind the Smad promoter DNA and control its activation.

By immunoelectron microscopy, we have shown internalization of the TGF-β1/Hyal-2 complex occurs via endosomes, followed by fusion of lysosomes with the internalized endosomes.[41] Whether degradation of TGF-β1

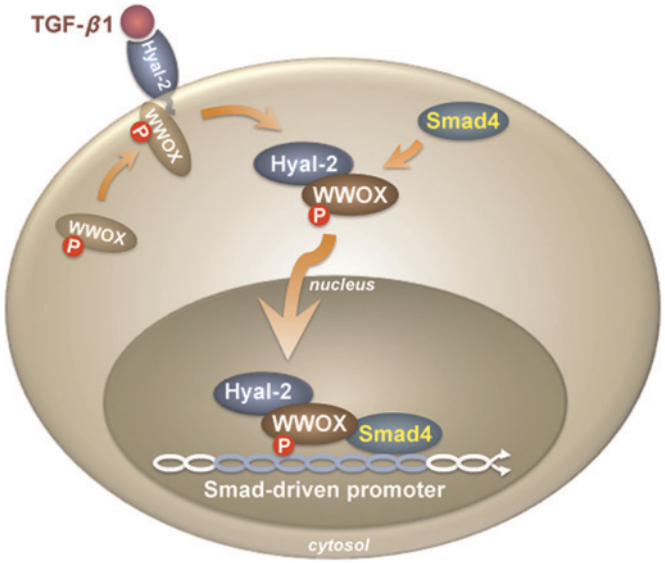

Figure 3 The TGF-β1/Hyal-2/WWOX/Smad signal pathway. Three likely signaling paths are: (i) TGF-β1 binds membrane Hyal-2, which subsequently recruits cytosolic Tyr33-phosphorylated WWOX (p-WWOX) to the membrane.[41] The resulting Hyal-2/p-WWOX complexes translocate to the nuclei for enhancing the Smad promoter activation. Overly activated Smad promoter induces cell death. (ii) Alternatively, the TGF-β1/Hyal-2 complexes are internalized via endocytic vesicles, followed by releasing of Hyal-2 to the cytoplasm for interacting with the cytosolic p-WWOX, and the Hyal-2/p-WWOX complexes re-locating to the nuclei. (iii) An additional scenario is that Smad4 is recruited to the Hyal-2/p-WWOX complexes, followed by re-locating to the nuclei. Ectopic WWOX and Smad4 dramatically increase the Smad promoter activation.[41] WWOX, WW domain-containing oxidoreductase; TGF, transforming growth factor; Hyal-2, hyaluronoglucosaminidase 2 (A color version of this figure is available in the online journal)

occurs in the lysosomes is unknown. Time-lapse FRET analysis in live cells revealed that WWOX binds Hyal-2 most strongly in the perinuclear area or on the nuclear envelope.[41] By co-immunoprecipitation and yeast two-hybrid analysis, Tyr33-phosphorylated WWOX binds effectively with Hyal-2, as a dominant-negative WWOX disrupts the binding.[41] Dominant-negative WWOX is known to prevent phosphorylation of WWOX at Tyr33.[37,38]

Functionally, Hyal-2 acts as a co-receptor with CD44 for hyaluronan. CD44 does not participate in the TGF-β1-induced nuclear translocation of WWOX.[41] Supporting evidence is that exogenous TGF-β1 does not interact with CD44, and cytosolic WWOX did not bind with CD44, as determined by co-immunoprecipitation and immunoelectron microscopy.[41] We propose that binding of TGF-β1 with Hyal-2 may be limited when CD44 and Hyal-2 are closely associated on the cell surface. However, upon TGF-β1 binding to Hyal-2, CD44 and Hyal-2 become dissociated.

An alternative scenario is that hyaluronan enhances the binding of TGF-β1 with Hyal-2 without transmitting the signal. Presumably, TGF-β1 is trapped on the cell surface by hyaluronan and Hyal-2. When hyaluronan is degraded, the signaling starts. Two reports showed that hyaluronan blocks TGF-β signaling by inducing trafficking of TGF-β receptors to lipid raft-associated pools, which facilitates increased receptor turnover.[84,85]

Sex steroid hormones (e.g. estrogen and androgen) are not able to induce nuclear accumulation of activated WWOX in estrogen receptor-positive breast MCF7 cells.[39] Presumably, Hyal-2 and Ezrin retain WWOX on the cell membrane/cytoskeleton areas and block its apoptotic function.

Complement protein C1q activates WWOX bypassing the classical pathway activation

Tissue fluids in the lung, for example, possess low levels of serum complement proteins. These proteins are important for self-defense and inflammatory reactions. The ion concentrations in tissue fluids are relatively low. Under these conditions, complement activation is enhanced to promote the inflammatory reaction.[86] Most recently, we discovered that specific serum complement components coordinate the activation of tumor suppressors p53 and WWOX and kinases extracellular signal-regulated kinase (ERK), JNK1 and signal transducer and activator of transcription 3 (STAT3) in cells.[40] In the absence of complement C1q or C6 in human serum samples, WWOX and ERK are mainly present in the cytoplasm without phosphorylation, whereas phosphorylated JNK1 is greatly accumulated in the nuclei. Reconstitution of C1q or C6 in serum restores the activation of WWOX and ERK, but blocks JNK1 activation. Exogenous C1q rapidly restores the WWOX activation in less than two hours. Without serum complement C9, p53 becomes activated. These observations suggest that each complement protein may play a specific role in modulating the activation of tumor suppressors and kinases.

We determined that complement C1q induces apoptosis of cancer cells overexpressing WWOX (Figure 4). The induced cell death is independent of the complement classical activation pathway. Without WWOX, C1q is not able to

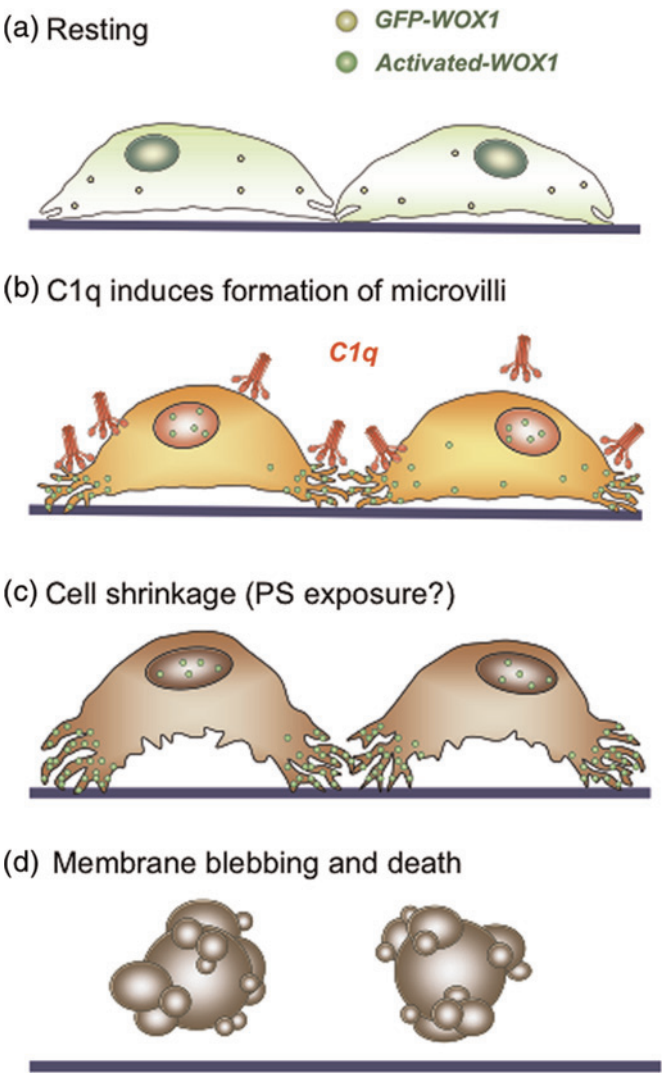

Figure 4 A sequential event of C1q-induced death of cells overexpressing WWOX. (a and b) Cells overexpressing WWOX adhere and spread evenly onto the surface of cover glass (determined by TIRF microscopy), whereas many cells may 'tiptoe' on the glass surface via formation of microvilli for focal adhesion.[40] Cells are colored in green for GFP-tagged WWOX. C1q dramatically increases the formation of microvilli as clusters in between cells. WWOX can be found in both the focal adhesion areas and in the nuclei. Cells are colored in orange, as they are agonized by C1q (b). (c and d) With time, the cells (brown color) undergo shrinkage, membrane blebbing, nuclear condensation and DNA fragmentation. Exposure of phosphatidylserine (PS) does not occur. C1q does not cause cell death, if endogenous levels of intracellular WWOX are low. WWOX, WW domain-containing oxidoreductase; TIRF, two-photon total internal reflection fluorescence; GFP, green fluorescent protein

cause apoptosis. Thus, these observations indicate the presence of a novel pathway of programmed cell death.

Imaging live C1q/WWOX signaling by surface plasmon-enhanced two-photon fluorescence microscopy

We utilized surface plasmon-enhanced two-photon total internal reflection fluorescence (TIRF) microscopy to investigate the dynamics of cell death initiating from the cell membrane.[87,88] Normally, cells adhere and spread smoothly on glass or plastic surface (Figure 4). Interestingly, many

WWOX-overexpressing cells adhere to glass surface by clusters of microvilli and their 'bellies' are up in the air without touching down on the glass surface. C1q further destabilizes the adherence of WWOX-expressing cells by increasing the formation of clustered microvilli for focal adhesion particularly in between cells. Time-lapse TIRF microscopy analysis revealed that these WWOX-rich microvilli are indeed 'dancing' upon C1q stimulation. Exposure of phosphatidylserine (PS) on the outer leaflet of the plasma membrane is a critical initiating event in apoptosis. However, membrane exposure of PS does not occur in WWOX-expressing cells in response to C1q. Ultimately, these cells undergo shrinkage, membrane blebbing and death. Overall, a novel pathway of WWOX-dependent programmed cell death is revealed.

Downregulation of WWOX and C1q in cancer cells

Many cancer tissues have very low levels of WWOX and C1q.[40] Under these conditions, cancer cells grow favorably. For example, benign prostatic hyperplasia and prostate cancer have a significantly reduced expression of tissue C1q, compared with age-matched normal prostate tissues.[40] By multitissue microarray analysis, we also determined that C1q is deficient in lung and many other cancer cells.

Conclusion and perspectives

Taken together, here we have summarized two newly discovered signaling events associated with WWOX as a downstream adaptor receiving signals from the cell surface. TGF-β1 binds cell surface hyaluronidase Hyal-2, followed by recruiting WWOX and Smad4 to control gene transcription, growth and death.[41] Complement C1q alone, bypassing the activation of the classical pathway, signals WWOX activation for apoptosis. This event occurs only when cells have sufficient amounts of intracellular WWOX.[40] The majority of cancer cells are deficient in WWOX, Smad4 and C1q. This could occur as a result of their progression toward cancerous phenotypes, thus gaining growth advantage. How the TGF-β/Hyal-2/WWOX/Smad4 signaling, coupled with the levels of hyaluronan and TGF-β, affects cancer progression *in vivo* remains to be assessed.

In addition to genetic alterations, metabolic turnover of WWOX appears to be critical in controlling cell growth. Phosphorylation of WWOX at Tyr287 by Ack1 leads to protein ubiquitination and degradation.[62] Interestingly, the E3 ubiquitin ligase NEDD4-2 downregulates Ack1,[63] which may in turn prolong the lifetime of WWOX. MDM2, an E3 ubiquitin ligase, binds WWOX.[38] Whether this binding promotes WWOX degradation remains to be established.

Complement C1q alone induces cancer death together with WWOX, bypassing the involvement of the components of the classical activation pathway. What remains unanswered is whether C1q receptor directly recruits or interacts with WWOX, or whether presence of an adaptor protein cross-links with WWOX and C1q receptor. C1q is known to interact with membrane proteins, including $\alpha 2\beta 1$ integrin,[89] gC1qR/p33,[90] C1q-Rp (CD93), cC1q-R (calreticulin) and CR1 (CD35).[91] The interaction between $\alpha 2\beta 1$ integrin and C1q plays an important role in the innate immune response.[89] Further, the connection with WWOX suggests the signaling is central to the homeostasis of immune cells, as well as normal cells.

Finally, many proteins are defined as tumor suppressors at the time when they are first discovered, simply because their functional deficiencies or genetic alterations lead the cells toward cancerous progression. Whether tumor suppressors always act as 'suppressors of growth' is not well supported. Indeed, essentially all tumor suppressors (e.g. p53, WWOX, Smad4 and so many others) are upregulated during cell mitosis. Their roles at this stage are largely unknown. Are they functionally active? Most ironically, tumor suppressor p53 plays a key role in the context of cell proliferation, differentiation, cell cycle, aging, metabolism and micro-RNA generation, and apoptosis.[92–95] Similarly, WWOX also plays a much bigger role than tumor suppression only.[16–21] WWOX is involved in maintaining normal cell physiology (e.g. mitochondrial functions), development of organs, bone metabolism, neural injury, apoptosis and others. WWOX is also an important sensor of environmental changes surrounding cells.

Author contributions: J-YC, H-PL, L-JH, F-JL, QH, S-JC, N-SC: writing and proof reading; J-YC, R-YH, H-PL, L-JH, S-JC, N-SC: graphs, discussions and critical supports for the manuscript.

ACKNOWLEDGEMENTS

Research was supported in part by the Department of Defense, USA (W81XWH-08-1-0682), the National Science Council, Taiwan (NSC96-2320-B-006-014, 96-2628-B-006-041-MY3 and 96-2628-B-006-045-MY3), the National Health Research Institute, Taiwan (NHRI-EX97-9704BI) and the National Cheng Kung University Landmark Projects (C0167 and R026) to N-SC, and the Chi-Mei Hospital and National Cheng Kung University Collaborative Research (CMNCKU9801) to F-JL and N-SC.

REFERENCES

1 Bork P, Sudol M. The WW domain: a signalling site in dystrophin? *Trends Biochem Sci* 1994;**19**:531–3
2 Sudol M, Chen HI, Bougeret C, Einbond A, Bork P. Characterization of a novel protein-binding module – the WW domain. *FEBS Lett* 1995;**369**:67–71
3 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. A map of WW domain family interactions. *Proteomics* 2004;**4**:643–55
4 Ingham RJ, Colwill K, Howard C, Dettwiler S, Lim CS, Yu J, Hersi K, Raaijmakers J, Gish G, Mbamalu G, Taylor L, Yeung B, Vassilovski G, Amin M, Chen F, Matskova L, Winberg G, Ernberg I, Linding R, O'Donnell P, Starostine A, Keller W, Metalnikov P, Stark C, Pawson T. WW domains provide a platform for the assembly of multiprotein networks. *Mol Cell Biol* 2005;**25**:7092–106
5 Kato Y, Nagata K, Takahashi M, Lian L, Herrero JJ, Sudol M, Tanokura M. Common mechanism of ligand recognition by group II/III WW domains: redefining their functional classification. *J Biol Chem* 2004;**279**:31833–41
6 Sudol M, Recinos CC, Abraczinskas J, Humbert J, Farooq A. WW or WoW: the WW domains in a union of bliss. *IUBMB Life* 2005;**57**:773–8

7 Wang K, Degerny C, Xu M, Yang XJ. YAP, TAZ, and Yorkie: a conserved family of signal-responsive transcriptional coregulators in animal development and human disease. *Biochem Cell Biol* 2009;**87**:77–91

8 Kanai F, Marignani PA, Sarbassova D, Yagi R, Hall RA, Donowitz M, Hisaminato A, Fujiwara T, Ito Y, Cantley LC, Yaffe MB. TAZ: a novel transcriptional co-activator regulated by interactions with 14-3-3 and PDZ domain proteins. *EMBO J* 2000;**19**:6778–91

9 Saucedo LJ, Edgar BA. Filling out the Hippo pathway. *Nat Rev Mol Cell Biol* 2007;**8**:613–21

10 Makita R, Uchijima Y, Nishiyama K, Amano T, Chen Q, Takeuchi T, Mitani A, Nagase T, Yatomi Y, Aburatani H, Nakagawa O, Small EV, Cobo-Stark P, Igarashi P, Murakami M, Tominaga J, Sato T, Asano T, Kurihara Y, Kurihara H. Multiple renal cysts, urinary concentration defects, and pulmonary emphysematous changes in mice lacking TAZ. *Am J Physiol Renal Physiol* 2008;**294**:F542–53

11 Sangadala S, Metpally RP, Reddy BV. Molecular interaction between Smurf1 WW2 domain and PPXY motifs of Smad1, Smad5, and Smad6 – modeling and analysis. *J Biomol Struct Dyn* 2007;**25**:11–23

12 Chen C, Matesic LE. The Nedd4-like family of E3 ubiquitin ligases and cancer. *Cancer Metast Rev* 2007;**26**:587–604

13 Behrens MI, Lendon C, Roe CM. A common biological mechanism in cancer and Alzheimer's disease? *Curr Alzheimer Res* 2009;**6**:196–204

14 Finn G, Lu KP. Phosphorylation-specific prolyl isomerase Pin1 as a new diagnostic and therapeutic target for cancer. *Curr Cancer Drug Targets* 2008;**8**:223–9

15 Esnault S, Shen ZJ, Malter JS. Pinning down signaling in the immune system: the role of the peptidyl-prolyl isomerase Pin1 in immune cell function. *Crit Rev Immunol* 2008;**28**:45–60

16 Chang NS. A potential role of p53 and WOX1 in mitochondrial apoptosis (review). *Int J Mol Med* 2002;**9**:19–24

17 Chang NS, Hsu LJ, Lin YS, Lai FJ, Sheu HM. WW domain-containing oxidoreductase: a candidate tumor suppressor. *Trends Mol Med* 2007;**13**:12–22

18 Smith DI, McAvoy S, Zhu Y, Perez DS. Large common fragile site genes and cancer. *Semin Cancer Biol* 2007;**17**:31–41

19 Aqeilan RI, Croce CM. WWOX in biological control and tumorigenesis. *J Cell Physiol* 2007;**212**:307–10

20 Yang J, Zhang W. WWOX tumor suppressor gene. *Histol Histopathol* 2008;**23**:877–82

21 Del Mare S, Salah Z, Aqeilan RI. WWOX: its genomics, partners, and functions. *J Cell Biochem* 2009;**108**:737–45

22 Lewandowska U, Zelazowski M, Seta K, Byczewska M, Pluciennik E, Bednarek AK. WWOX, the tumour suppressor gene affected in multiple cancers. *J Physiol Pharmacol* 2009;**1**(Suppl. 60):47–56

23 Sze CI, Su M, Pugazhenthi S, Jambal P, Hsu LJ, Heath J, Schultz L, Chang NS. Down-regulation of WW domain-containing oxidoreductase induces Tau phosphorylation *in vitro*. A potential role in Alzheimer's disease. *J Biol Chem* 2004;**279**:30498–506

24 Chen ST, Chuang JI, Wang JP, Tsai MS, Li H, Chang NS. Expression of WW domain-containing oxidoreductase WOX1 in the developing murine nervous system. *Neuroscience* 2004;**124**:831–9

25 Chen ST, Chuang JI, Cheng CL, Hsu LJ, Chang NS. Light-induced retinal damage involves tyrosine 33 phosphorylation, mitochondrial and nuclear translocation of WW domain-containing oxidoreductase *in vivo*. *Neuroscience* 2005;**130**:397–407

26 Lo CP, Hsu LJ, Li MY, Hsu SY, Chuang JI, Tsai MS, Lin SR, Chang NS, Chen ST. MPP^+-induced neuronal death in rats involves tyrosine 33 phosphorylation of WW domain-containing oxidoreductase WOX1. *Eur J Neurosci* 2008;**27**:1634–46

27 Li MY, Lai FJ, Hsu LJ, Lo CP, Cheng CL, Tsai MS, Lin SR, Lee MH, Chang JY, Subhan D, Sze CI, Pugazhenthi S, Chang NS, Chen ST. Dramatic co-activation of WOX1 with CREB and NF-κB in delayed loss of small dorsal root ganglion neurons upon sciatic nerve transection in rats. *PLoS One* 2009;**4**:e7820

28 Aqeilan RI, Hassan MQ, de Bruin A, Hagan JP, Volinia S, Palumbo T, Hussain S, Lee SH, Gaur T, Stein GS, Lian JB, Croce CM. The WWOX tumor suppressor is essential for postnatal survival and normal bone metabolism. *J Biol Chem* 2008;**283**:21629–39

29 Ludes-Meyers JH, Kil H, Parker-Thornburg J, Kusewitt DF, Bedford MT, Aldaz CM. Generation and characterization of mice carrying a conditional allele of the Wwox tumor suppressor gene. *PLoS One* 2009;**4**:e7775

30 Ludes-Meyers JH, Kil H, Nunez MI, Conti CJ, Parker-Thornburg J, Bedford MT, Aldaz CM. WWOX hypomorphic mice display a higher incidence of B-cell lymphomas and develop testicular atrophy. *Genes Chromosomes Cancer* 2007;**46**:1129–36

31 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. Targeted ablation of the WW domain-containing oxidoreductase tumor suppressor leads to impaired steroidogenesis. *Endocrinology* 2009;**150**:1530–5

32 Iliopoulos D, Malizos KN, Oikonomou P, Tsezou A. Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One* 2008;**3**:e3740

33 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. WW-domain-containing oxidoreductase is associated with low plasma HDL-C levels. *Am J Hum Genet* 2008;**83**:180–92

34 Suzuki H, Katayama K, Takenaka M, Amakasu K, Saito K, Suzuki K. A spontaneous mutation of the Wwox gene and audiogenic seizures in rats with lethal dwarfism and epilepsy. *Genes Brain Behav* 2009;**8**:650–60

35 Chang NS, Pratt N, Heath J, Schultz L, Sleve D, Carey GB, Zevotek N. Hyaluronidase induction of a WW domain-containing oxidoreductase that enhances tumor necrosis factor cytotoxicity. *J Biol Chem* 2001;**276**:3361–70

36 Lai FJ, Cheng CL, Chen ST, Wu CH, Hsu LJ, Lee JY, Chao SC, Sheen MC, Shen CL, Chang NS, Sheu HM. WOX1 is essential for UVB irradiation-induced apoptosis and down-regulated via translational blockade in UVB-induced cutaneous squamous cell carcinoma *in vivo*. *Clin Cancer Res* 2005;**11**:5769–77

37 Chang NS, Doherty J, Ensign A. JNK1 physically interacts with WW domain-containing oxidoreductase (WOX1) and inhibits WOX1-mediated apoptosis. *J Biol Chem* 2003;**278**:9195–202

38 Chang NS, Doherty J, Ensign A, Schultz L, Hsu LJ, Hong Q. WOX1 is essential for tumor necrosis factor-, UV light-, staurosporine-, and p53-mediated cell death, and its tyrosine 33-phosphorylated form binds and stabilizes serine 46-phosphorylated p53. *J Biol Chem* 2005;**280**:43100–8

39 Chang NS, Schultz L, Hsu LJ, Lewis J, Su M, Sze CI. 17beta-Estradiol upregulates and activates WOX1/WWOXv1 and WOX2/WWOXv2 *in vitro*: potential role in cancerous progression of breast and prostate to a premetastatic state *in vivo*. *Oncogene* 2005;**24**:714–23

40 Hong Q, Sze CI, Lin SR, Lee MH, He RY, Schultz L, Chang JY, Chen SJ, Boackle RJ, Hsu LJ, Chang NS. Complement C1q activates tumor suppressor WWOX to induce apoptosis in prostate cancer cells. *PLoS One* 2009;**4**:e5755

41 Hsu LJ, Schultz L, Hong Q, Van Moer K, Heath J, Li MY, Lai FJ, Lin SR, Lee MH, Lo CP, Lin YS, Chen ST, Chang NS. Transforming growth factor beta1 signaling via interaction with cell surface Hyal-2 and recruitment of WWOX/WOX1. *J Biol Chem* 2009;**284**:16049–59

42 Aqeilan RI, Pekarsky Y, Herrero JJ, Palamarchuk A, Letofsky J, Druck T, Trapasso F, Han SY, Melino G, Huebner K, Croce CM. Functional association between Wwox tumor suppressor protein and p73, a p53 homolog. *Proc Natl Acad Sci USA* 2004;**101**:4401–6

43 Jin C, Ge L, Ding X, Chen Y, Zhu H, Ward T, Wu F, Cao X, Wang Q, Yao X. PKA-mediated protein phosphorylation regulates ezrin-WWOX interaction. *Biochem Biophys Res Commun* 2006;**341**:784–91

44 Bednarek AK, Laflin KJ, Daniel RL, Liao Q, Hawkins KA, Aldaz CM. WWOX, a novel WW domain-containing protein mapping to human chromosome 16q23.3–24.1, a region frequently affected in breast cancer. *Cancer Res* 2000;**60**:2140–5

45 Ried K, Finnis M, Hobson L, Mangelsdorf M, Dayan S, Nancarrow JK, Woollatt E, Kremmidiotis G, Gardner A, Venter D, Baker E, Richards RI. Common chromosomal fragile site FRA16D sequence: identification of the FOR gene spanning FRA16D and homozygous deletions and translocation breakpoints in cancer cells. *Hum Mol Genet* 2000;**9**:1651–63

46 Ludes-Meyers JH, Bednarek AK, Popescu NC, Bedford M, Aldaz CM. WWOX, the common chromosomal fragile site, FRA16D, cancer gene. *Cytogenet Genome Res* 2003;**100**:101–10

47 Matsuyama A, Croce CM, Huebner K. Common fragile genes. *Eur J Histochem* 2004;**48**:29–36

48 Ishii H, Furukawa Y. Alterations of common chromosome fragile sites in hematopoietic malignancies. *Int J Hematol* 2004;**79**:238–42
49 O'Keefe LV, Richards RI. Common chromosomal fragile sites and cancer: focus on FRA16D. *Cancer Lett* 2006;**232**:37–47
50 Aqeilan RI, Trapasso F, Hussain S, Costinean S, Marshall D, Pekarsky Y, Hagan JP, Zanesi N, Kaou M, Stein GS, Lian JB, Croce CM. Targeted deletion of Wwox reveals a tumor suppressor function. *Proc Natl Acad Sci USA* 2007;**104**:3949–54
51 Ilsley JL, Sudol M, Winder SJ. The WW domain: linking cell signalling to the membrane cytoskeleton. *Cell Signal* 2002;**14**:183–9
52 Lapan P, Zhang J, Hill A, Zhang Y, Martinez R, Haney S. Image-based assessment of growth and signaling changes in cancer cells mediated by direct cell-cell contact. *PLoS One* 2009;**4**:e6822
53 Li Y, Zhou Z, Alimandi M, Chen C. WW domain containing E3 ubiquitin protein ligase 1 targets the full-length ErbB4 for ubiquitin-mediated degradation in breast cancer. *Oncogene* 2009;**28**:2948–58
54 Lokeshwar VB, Cerwinka WH, Isoyama T, Lokeshwar BL. HYAL1 hyaluronidase in prostate cancer: a tumor promoter and suppressor. *Cancer Res* 2005;**65**:7782–89
55 Aqeilan RI, Palamarchuk A, Weigel RJ, Herrero JJ, Pekarsky Y, Croce CM. Physical and functional interactions between the Wwox tumor suppressor protein and the AP-2gamma transcription factor. *Cancer Res* 2004;**64**:8256–61
56 Aqeilan RI, Donati V, Palamarchuk A, Trapasso F, Kaou M, Pekarsky Y, Sudol M, Croce CM. WW domain-containing proteins, WWOX and YAP, compete for interaction with ErbB-4 and modulate its transcriptional function. *Cancer Res* 2005;**65**:6764–72
57 Aqeilan RI, Donati V, Gaudio E, Nicoloso MS, Sundvall M, Korhonen A, Lundin J, Isola J, Sudol M, Joensuu H, Croce CM, Elenius K. Association of Wwox with ErbB4 in breast cancer. *Cancer Res* 2007;**67**:9330–6
58 Ludes-Meyers JH, Kil H, Bednarek AK, Drake J, Bedford MT, Aldaz CM. WWOX binds the specific proline-rich ligand PPXY: identification of candidate interacting proteins. *Oncogene* 2004;**23**:5049–55
59 Gaudio E, Palamarchuk A, Palumbo T, Trapasso F, Pekarsky Y, Croce CM, Aqeilan RI. Physical association with WWOX suppresses c-Jun transcriptional activity. *Cancer Res* 2006;**66**:11585–9
60 Hong Q, Hsu LJ, Schultz L, Pratt N, Mattison J, Chang NS. Zfra affects TNF-mediated cell death by interacting with death domain protein TRADD and negatively regulates the activation of NF-kappaB, JNK1, p53 and WOX1 during stress response. *BMC Mol Biol* 2007;**8**:50
61 Hsu LJ, Schultz L, Mattison J, Lin YS, Chang NS. Cloning and characterization of a small-size peptide Zfra that regulates the cytotoxic function of tumor necrosis factor by interacting with JNK1. *Biochem Biophys Res Commun* 2005;**327**:415–23
62 Mahajan NP, Whang YE, Mohler JL, Earp HS. Activated tyrosine kinase Ack1 promotes prostate tumorigenesis: role of Ack1 in polyubiquitination of tumor suppressor Wwox. *Cancer Res* 2005;**65**:10514–23
63 Chan W, Tian R, Lee YF, Sit ST, Lim L, Manser E. Down-regulation of active ACK1 is mediated by association with the E3 ubiquitin ligase Nedd4-2. *J Biol Chem* 2009;**284**:8185–94
64 Yang G, Zhang G, Pittelkow MR, Ramoni M, Tsao H. Expression profiling of UVB response in melanocytes identifies a set of p53-target genes. *J Invest Dermatol* 2006;**126**:2490–506
65 Komuro A, Imamura T, Saitoh M, Yoshida Y, Yamori T, Miyazono K, Miyazawa K. Negative regulation of transforming growth factor-beta (TGF-beta) signaling by WW domain-containing protein 1 (WWP1). *Oncogene* 2004;**23**:6914–23
66 Morén A, Imamura T, Miyazono K, Heldin CH, Moustakas A. Degradation of the tumor suppressor Smad4 by WW and HECT domain ubiquitin ligases. *J Biol Chem* 2005;**280**:22115–23
67 Kuratomi G, Komuro A, Goto K, Shinozaki M, Miyazawa K, Miyazono K, Imamura T. NEDD4-2 (neural precursor cell expressed, developmentally down-regulated 4-2) negatively regulates TGF-beta (transforming growth factor-beta) signalling by inducing ubiquitin-mediated degradation of Smad2 and TGF-beta type I receptor. *Biochem J* 2005;**386**:461–70
68 Massagué J. TGFbeta in Cancer. *Cell* 2008;**134**:215–30
69 Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. *Cell Res* 2009;**19**:156–72
70 Muraoka-Cook RS, Dumont N, Arteaga CL. Dual role of transforming growth factor beta in mammary tumorigenesis and metastatic progression. *Clin Cancer Res* 2005;**11**:937s–43s
71 Bachman KE, Park BH. Duel nature of TGF-beta signaling: tumor suppressor vs. tumor promoter. *Curr Opin Oncol* 2005;**17**:49–54
72 Munoz NM, Upton M, Rojas A, Washington MK, Lin L, Chytil A, Sozmen EG, Madison BB, Pozzi A, Moon RT, Moses HL, Grady WM. Transforming growth factor beta receptor type II inactivation induces the malignant transformation of intestinal neoplasms initiated by Apc mutation. *Cancer Res* 2006;**66**:9837–44
73 Kitamura T, Taketo MM. Keeping out the bad guys: gateway to cellular target therapy. *Cancer Res* 2007;**67**:10099–102
74 Yang L, Huang J, Ren X, Gorska AE, Chytil A, Aakre M, Carbone DP, Matrisian LM, Richmond A, Lin PC, Moses HL. Abrogation of TGF beta signaling in mammary carcinomas recruits Gr-1+CD11b+ myeloid cells that promote metastasis. *Cancer Cell* 2008;**13**:23–35
75 Engle SJ, Hoying JB, Boivin GP, Ormsby I, Gartside PS, Doetschman T. Transforming growth factor beta1 suppresses nonmetastatic colon cancer at an early stage of tumorigenesis. *Cancer Res* 1999;**59**:3379–86
76 Bacman D, Merkel S, Croner R, Papadopoulos T, Brueckl W, Dimmler A. TGF-beta receptor 2 downregulation in tumour-associated stroma worsens prognosis and high-grade tumours show more tumour-associated macrophages and lower TGF-beta1 expression in colon carcinoma: a retrospective study. *BMC Cancer* 2007;**7**:156
77 Stern R. Association between cancer and 'acid mucopolysaccharides': an old concept comes of age, finally. *Semin Cancer Biol* 2008;**18**:238–43 (review)
78 Simpson MA, Lokeshwar VB. Hyaluronan and hyaluronidase in genitourinary tumors. *Front Biosci* 2008;**13**:5664–80
79 Cattaruzza S, Perris R. Proteoglycan control of cell movement during wound healing and cancer spreading. *Matrix Biol* 2005;**24**:400–17
80 Chang NS. Hyaluronidase enhancement of TNF-mediated cell death is reversed by TGF-beta 1. *Am J Physiol* 1997;**273**:C1987–94
81 Chang NS. Transforming growth factor-beta protection of cancer cells against tumor necrosis factor cytotoxicity is counteracted by hyaluronidase (review). *Int J Mol Med* 1998;**2**:653–59
82 Chang NS. Transforming growth factor-beta1 blocks the enhancement of tumor necrosis factor cytotoxicity by hyaluronidase Hyal-2 in L929 fibroblasts. *BMC Cell Biol* 2002;**3**:8
83 Chang NS. Transforming growth factor-beta 1 induction of novel extracellular matrix proteins that trigger resistance to tumor necrosis factor cytotoxicity in murine L929 fibroblasts. *J Biol Chem* 1995;**270**:7765–72
84 Ito T, Williams JD, Fraser DJ, Phillips AO. Hyaluronan regulates transforming growth factor-beta1 receptor compartmentalization. *J Biol Chem* 2004;**279**:25326–32
85 Ito T, Williams JD, Fraser D, Phillips AO. Hyaluronan attenuates transforming growth factor-beta1-mediated signaling in renal proximal tubular epithelial cells. *Am J Pathol* 2004;**164**:1979–88
86 Chang NS, Boackle RJ. Unusual complement-mediated hemolytic kinetics at low ionic strength. *Mol Immunol* 1985;**22**:1217–24
87 He RY, Su YD, Cho KC, Lin CY, Chang NS, Chang CH, Chen SJ. Surface plasmon-enhanced two-photon fluorescence microscopy for live cell membrane imaging. *Opt Expr* 2009;**17**:5987–97
88 He RY, Su YD, Chiu KC, Chang NS, Wu HL, Chen SJ. Imaging live cell membranes via surface plasmon-enhanced fluorescence and phase microscopy. *Opt Expr* 2010;**18**:3649–59
89 Zutter MM, Edelson BT. The alpha2beta1 integrin: a novel collectin/C1q receptor. *Immunobiology* 2007;**212**:343–53
90 Peerschke EI, Ghebrehiwet B. The contribution of gC1qR/p33 in infection and inflammation. *Immunobiology* 2007;**212**:333–42
91 Ghebrehiwet B, Peerschke EI. cC1q-R (calreticulin) and gC1q-R/p33: ubiquitously expressed multi-ligand binding cellular proteins involved in inflammation and infection. *Mol Immunol* 2004;**41**:173–83
92 Junttila MR, Evan GI. p53 – a Jack of all trades but master of none. *Nat Rev Cancer* 2009;**9**:821–9
93 Speidel D. Transcription-independent p53 apoptosis: an alternative route to death. *Trends Cell Biol* 2010;**20**:14–24
94 Takwi A, Li Y. The p53 apthway encounters the microRNA world. *Curr Genom* 2009;**10**:194–7
95 Feng Z, Hu W, Rajagopal G, Levine AJ. The tumor suppressor p53: cancer and aging. *Cell Cycle* 2008;**7**:842–7
96 Watanabe A, Hippo Y, Taniguchi H, Iwanari H, Yashiro M, Hirakawa K, Kodama T, Aburatani H. An opposing view on WWOX protein function as a tumor suppressor. *Cancer Res* 2003;**63**:8629–33