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Molecular mechanisms underlying WOX1 activation during apoptotic and stress responses

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Abstract

Human *WWOX* gene encodes a putative tumor suppressor WW domain-containing oxidoreductase WOX1 (also known as WWOX or FOR). A high frequency of loss of heterozygosity (LOH) of this gene has been shown in prostate, lung, breast and other cancers. In addition, numerous aberrant *WWOX* mRNA transcripts have been found in cancer cells. WOX1 is a proapoptotic protein. In response to stress or apoptotic stimuli, WOX1 became phosphorylated at Tyr33, which enabled its complex formation with activated p53 and JNK1. The p53/WOX1 complex translocated to the mitochondria and further to the nuclei to mediate apoptosis. WOX1 mutants, which were inactivated for nuclear translocation or Tyr33 phosphorylation, failed to induce apoptosis, indicating that activation of WOX1 via Tyr33 phosphorylation, followed by nuclear translocation, is essential for inducing cell death. WOX1 induced apoptosis synergistically with p53. In contrast, transiently activated JNK1 induced anti-apoptotic response, and this protective activity inhibited WOX1-induced apoptosis. Taken together, WOX1 is involved in stress and apoptotic responses, and is likely to regulate the activation of both p53 and JNK1.

Keywords: WOX1; TNF; p53; JNK1; Apoptosis; ADH/SDR

1. WOX1/WWOX/FOR: a putative tumor suppressor

Tumorigenesis may occur as a consequence of sequential and accumulative mutations and/or deletions of certain genes [1,2]. Inactivation of tumor suppressor function of genes such as *p53* and *BRCA1* predisposes cells to neoplastic growth [3,4]. Human *WWOX* gene is located on a chromosomal fragile site ch16q23.3-24.1 [5] or ch16q23.2 [6], which spans the common fragile site FRA16D. Alteration of this gene has been demonstrated in multiple types of cancers [5–10]. High frequency of loss of heterozygosity (LOH) of ch16q23.3-24.1 chromosome (30–55%) has been shown in hepatocellular carcinoma [7], ovarian [8], breast [9], esophageal [10], prostate [11,12], and lung cancer [13]. However, mutations in the *WWOX* gene are relatively uncommon. *FHIT* gene is also located on a chromosomal fragile site, ch3p14.2 [14]. Inactivation of both *WWOX* and *FHIT* genes involves breakage at the fragile sites, rather than point mutations. In addition, multiple mRNA transcripts of these genes are frequently observed in cancer cells.

The *WWOX* gene encodes a putative tumor suppressor WOX1; also known as WWOX or FOR. We have shown that WOX1 is a proapoptotic protein [15–17]. Another study showed that WOX1 can act as a tumor suppressor both *in vitro* and *in vivo* [18]. This review summarizes the present knowledge on this novel mediator of apoptotic and stress response pathways, by presenting the recently determined interactions with p53, JNK1 and others in various signaling cascades. Furthermore, we outline several other biologically important areas where WOX1-mediated and -integrated pathways would be of physiologic significance.

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Abbreviations: WOX1 (or WWOX), WW domain-containing oxidoreductase; NLS, nuclear localization sequence; JNK1, c-Jun-N-terminal kinase; ADH, alcohol dehydrogenase; SDR, short-chain dehydrogenase/reductase; TNF, tumor necrosis factor; TRADD, TNF receptor-associated death domain protein; TRAF2, TNF receptor-associated factor 2; AIF, apoptosis-inducing factor.

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2. WOX1/WWOX/FOR and family proteins

We isolated murine WOX1 by functional cloning [15]. Other laboratories have concurrently isolated the human *WWOX* gene and cDNA clones [5,6]. The full-length WOX1 (46 kDa) possesses two N-terminal WW domains (containing conserved tryptophan residues), a NLS, and a C-terminal short-chain ADH or SDR domain (Fig. 1). The *WWOX* gene has nine exons. Seven members of the human WOX protein family have been found, as reported in the Genbank database (Table 1). The ADH domain region, which is encoded by exons 4–8, is frequently deleted or alternatively spliced. In contrast, the WW domain region is rarely deleted, and its amino acid sequence is highly conserved among human, mouse and rat. Interestingly, the WW domain region is different in *Drosophila* [10]. Due to the alternative splicing in the ADH domain, essentially every WOX protein possesses a unique C-terminal amino acid sequence, with few exceptions (Fig. 1 and Table 1). We have recently isolated a 60-kDa WOX variant, designated WOX8 (or v8), which possesses an elongated C terminus identical to that of the v3 variant (see Table 1 and Fig. 1; Chang *et al.*, unpublished). The elongation is caused by deletion of two bases in the DNA sequence. This protein can be identified in Western blotting; however, its functional property remains to be determined.

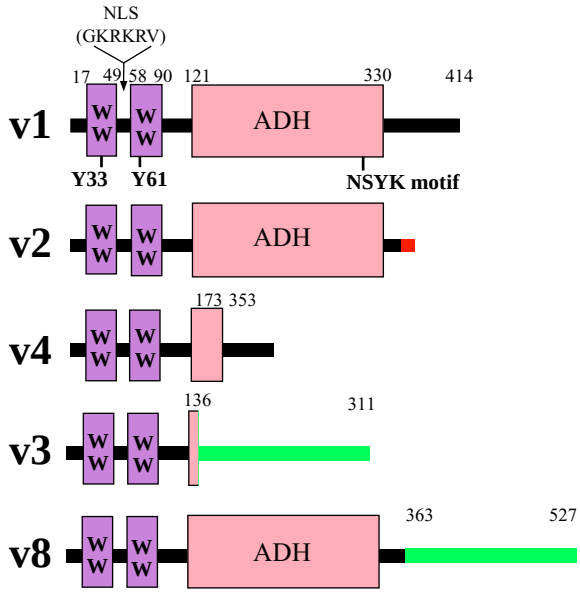

Fig. 1. Schematic structures of WOX1 and four representative variants. The amino acid sequence (414 amino acids) of WOX1 (WWOX, FOR, or v1) possesses two N-terminal WW domains, a nuclear localization signal sequence (NLS), and a C-terminal short-chain alcohol dehydrogenase (ADH) domain. A mitochondrion-targeting sequence in WOX1 was mapped within the ADH domain (amino acid #209–273) [15]. The Tyr33 phosphorylation site is indicated [16]. The potential of Tyr61 phosphorylation is unknown. The conserved catalytic tetrad N-S-Y-K motif is shown (N232, S281, Y293, K297). v2 possesses a unique C terminus (red). v4 has a deletion in the ADH domain (merged sites indicated), but possesses an identical C terminus as that of the wild type WOX1 or v1 (black). Both v3 and v8 have an identical C terminus (green); however, v3 does not have an intact ADH domain.

3. The SDR/ADH domain in WOX1 and its potential ligands

The protein family of the SDR (or ADH) domain constitutes more than 2000 proteins [19]. The SDR enzymes are normally 250–300 amino acid residues in length, possessing a catalytic triad of Ser-Tyr-Lys motif and a nucleotide-binding motif Gly-X_3-Gly-X-Gly in the sequence. An additional active site residue Asn has recently been identified to form a catalytic tetrad of Asn-Ser-Tyr-Lys (N-S-Y-K) in the majority of the SDR proteins [20]. By deletion analysis, we have identified the presence of a mitochondria-targeting region in the ADH domain of murine WOX1 [15]. Modifications in the N or C termini such as additions with WW domains, signal peptides, or transmembrane domains have been found in the SDR proteins [19]. The enzymatic functions of SDR proteins are related with oxidation/reduction of lipid hormones and metabolic mediators [21]. For instance, 17β-hydroxysteroid dehydrogenases are evolutionally conserved enzymes responsible for the physiological

Table 1
Human WOX1/WWOX/FOR and family proteins[a]

Name	aa #	Size (kDa)	NSYK[b]	Modifications[c]	Last 15 aa at C terminus	Other names
v1	424	46	Yes	Wild type	LSERLIQERLGSQSG[d]	WOX1; FOR2; WWOX
v2	363	41	Yes	Exon 9 partial del.	TKSMVSDCLVEGGHF	WOX2; FOR1
v3	311	35	No	Exons 5–8 del.	EKHQQFSFFYCYRIA[e]	
v4	234	26	No	Exons 6–8 del.	LSERLIQERLGSQSG[d]	
v5	213	24	No	Exons 5–9 del.	RAKRRPGPCGRSARG	
v6	189	22	No	Exons 6–9 del.	YHPPPEKCRIKIFH	WOX3; FOR3
v7	36	4	No	Exons 2–9 del.	WEERTTKDGWVYYAK	
v8	527	59	Yes	Exon 9, 2 bases del.	EKHQQFSFFYCYRIA[e]	WOX8

[a] v1–v7 from published Genbank database. v8 from Chang *et al.*, unpublished.
[b] A catalytic tetrad NSYK motif for binding with substrates such as estrogen and androgen.
[c] Alternative splicing and deletion.
[d] Identical C terminus.
[e] Identical C terminus.

responses of androgens and estrogens [22,23]. At least 10 different isoforms of 17β-hydroxysteroid dehydrogenases of the SDR family have been defined at present [23].

Accordingly, one of the likely functions of WOX1 is its involvement in the metabolism of steroid hormones such as androgens and estrogens. Although the mRNA levels of WOX1 and its variants have been determined in a variety of cancers by RT/PCR [5–10], the protein levels have never been determined in cancerous tissues and organs. By utilizing specific antibodies and tissue microarray analyses, we have demonstrated a significant elevation of WOX1 protein levels in breast and prostate cancers (Lewis and Chang, unpublished). While development of these cancers is mostly hormone-dependent [24,25], it is suggested that the elevated WOX1 is needed for the metabolism of steroid hormones. Most interestingly, the protein levels of WOX3 (or v6; see Table 1) are also significantly increased in these cancers (Lewis and Chang, unpublished). WOX3 has a defective ADH domain and is unlikely to metabolize steroid hormones. Upregulation of WOX3 in breast and prostate cancers may be essential for the transition of hormone dependence to independence during their development.

4. WOX1 localization and translocation to mitochondria and nuclei

WOX1 is a proapoptotic protein, capable of interacting with p53 [15,26,27]. The functional characteristics of murine and human WOX1 are summarized in Table 2. A portion of endogenous WOX1 protein is located in the mitochondria (as verified using purified rat liver mitochondria), and the mitochondrion-targeting sequence has been mapped within the ADH domain [15]. During apoptosis or stress responses, there is an increased synthesis of cytosolic WOX1, followed by translocating to the mitochondria [26]. A recent report showed that ectopic human GFP-WOX1 is present in the Golgi complex but not in the mitochondria [18]. However, in this study, Golgi complex and mitochondria were not purified to ensure the presence or absence of WOX1 in these organelles. We have re-examined endogenous WOX1 localization by using three newly generated N-terminal and one C-terminal antibodies against different regions in WOX1. Also, antibodies against phospho-Tyr33 WOX1 were used [16]. Again, we found the localization of WOX1 in the mitochondria in several tested cell lines and primary human fibroblasts. Most conclusively, by electron microscopy we have demonstrated an increased presence of WOX1 in the damaged mitochondria and condensed nuclei of retinal degenerating photoreceptors in rats (Chen *et al.*, unpublished).

Ectopic expression of the murine wild type WOX1, tagged with either EGFP or ECFP, induces clustering of mitochondria in a majority of cultured cells, except in neonatal rat heart H9c2 cardiomyocytes. The overexpressed WOX1 distributes evenly in the cytoplasm of H9c2 cells. TNF induces translocation of the ectopic WOX1 protein to the mitochondria. Induction of mitochondrial permeability transition by TNF, staurosporine, and atractyloside results in WOX1 release from the mitochondria and subsequent nuclear translocation [15]. TNF-mediated WOX1 nuclear translocation occurs shortly after that of NF-κB [15].

The ADH domain may affect the intracellular localization of WOX1. Ectopic expression of the ADH-defective WOX1 variants may result in nuclear presence of these proteins. The nuclear localization sequence is located between the first and second WW domain (Fig. 1). Alteration of this sequence by site-directed mutagenesis abolishes nuclear translocation of WOX1 [15].

5. WOX1 enhances TNF cytotoxicity

WOX1 enhances TNF cytotoxicity in L929 fibroblasts via its WW and ADH domains as determined using stable

Table 2
Functional properties of WOX1

	References
Hyaluronidases PH-20, Hyal-1 and Hyal-2 induce WOX1 expression	[15,17]
Presence of WOX1 protein in mitochondria, as determined by light and electron microscopy[a]	[15,26]
Induction of mitochondrial permeability transition leads to translocation of WOX1 to nucleus	[15,26]
WOX1 enhances TNF cytotoxicity by downregulating Bcl-2 and Bcl-xL, but upregulating p53	[15]
Overexpressed WOX1 induces apoptosis synergistically with p53	[15,16]
The WW domains of WOX1 physically interact with the poly-proline domain and phospho-Ser46 in p53[b]	[15]
Blocking of WOX1 expression by antisense or dominant negative WOX1 abolishes p53 apoptosis	[15,16]
Phosphorylation of Tyr33 in the first WW domain is essential for p53/WOX1 interaction	[16]
Hyal-2 enhances WOX1-induced apoptosis	[17]
Tyr33-phosphorylated WOX1 physically interacts with activated JNK1 under stress conditions	[16]
Ectopic JNK1 blocks WOX1-induced inhibition of cell cycle progression and apoptosis	[16]
Ectopic WOX1 acts as a tumor suppressor *in vivo* and *in vitro*	[15,18]

[a] Observations with electron microscopy (Chen *et al.*, submitted).
[b] Chang *et al.*, unpublished.

cell transfectants [15]. Upregulation of the proapoptotic p53 and downregulation of the apoptosis inhibitors Bcl-2 and Bcl-xL are involved in WOX1-increased TNF cytotoxic function. WOX1 also enhances ectopic TRADD-mediated cell death [15]. Accordingly, antisense expression of WOX1 raises TNF resistance in L929 cells [15].

WOX1 significantly enhances the apoptotic function of ectopic TRADD [15], indicating a potential binding of WOX1 with TRADD during TNF signaling. To explore the possible mechanisms, we determined that WOX1 physically interacts with TRADD and TRAF2 of the TNF signaling pathway using two-hybrid analysis (Fig. 2). The WOX1/TRAF2 interaction in cultured cells has been further confirmed by co-immunoprecipitation (Chang *et al.*, unpublished), while the WOX1/TRADD interaction remains to be further confirmed. Presumably, WOX1 enhancement of TRADD cytotoxic function is due to their binding interactions (Fig. 2; a detailed TNF signaling pathway). In contrast, TRAF2 is involved in the protective or survival pathway of TNF signaling that activates NF-κB. Mechanisms by which WOX1 blocks TRAF2-mediated cell survival or increases TRADD-mediated apoptosis remain to be established.

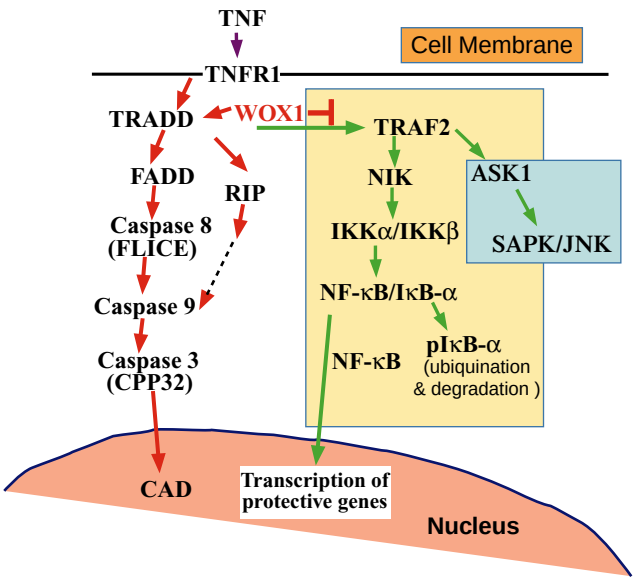

Fig. 2. WOX1 enhances TNF apoptosis by interacting with TRADD and TRAF2. TNF mediates both apoptotic (red arrows) and anti-apoptotic (green arrows) pathways. TNF binds to the membrane p55 TNFR1, which immediately recruits TRADD and FADD (TNF receptor or Fas-associated death domain protein) and activates caspase 8, which in turn activates caspase 9, caspase 3 and downstream caspase 6 and 7. Alternatively, TNFR1/TRADD binds RIP (receptor interacting protein) and activates caspase 3. Caspase 3 cleaves structural proteins and activates a specific caspase-activated DNase (CAD) that mediates nuclear DNA fragmentation, thus leading to cell death. In the protective pathway (yellow box), TNFR1/TRADD recruits TRAF2 that leads to NF-κB activation. NF-κB appears to transcribe protective proteins that block cell death. In a second link, TRAF2 activates apoptosis signal-regulating kinase 1 (ASK1), which in turn activates SAPK/JNK (stress activate protein kinase/c-Jun N-terminal kinase; blue box). WOX1 enhances TRADD-mediated apoptosis [15]. We found that WOX1 binds TRADD and TRAF2 in yeast two-hybrid library screening, and WOX1/TRAF2 binding was further confirmed by co-immunoprecipitation. The effect of these protein interactions in regulating TNF signaling remains to be established.

6. Tyr33 phosphorylation and nuclear translocation are needed for WOX1-mediated apoptosis

When overexpressed, the full-length or the WW-domain region of WOX1 induces apoptosis [15–17]. Alteration of Tyr33 to Arg33 in the first WW domain results in abrogation of WOX1-mediated cell death [16]. To determine whether Tyr33 undergoes phosphorylation during apoptosis and stress responses, we have produced specific antibodies against a synthetic phospho-Tyr33 peptide corresponding to the first WW-domain sequence [16]. To induce stress response, cultured cells were treated with anisomycin to activate JNK1. We found that anisomycin induces activation or phosphorylation of JNK1, as well as phosphorylation of WOX1 at Tyr33. Similarly, UV light-mediated stress response involves phosphorylation of WOX1, p53, and JNK1. The phosphorylated WOX1 can be considered as "activated" and is found mostly in the mitochondria (Chang *et al.*, unpublished), suggesting that WOX1 phosphorylation is essential for its translocation to the mitochondria from cytoplasm (Fig. 3). Whether the activated WOX1 is responsible for mitochondrial clustering is unknown at present.

Depending upon the strength of exogenous or endogenous stress or apoptotic signals, the activated WOX1 further

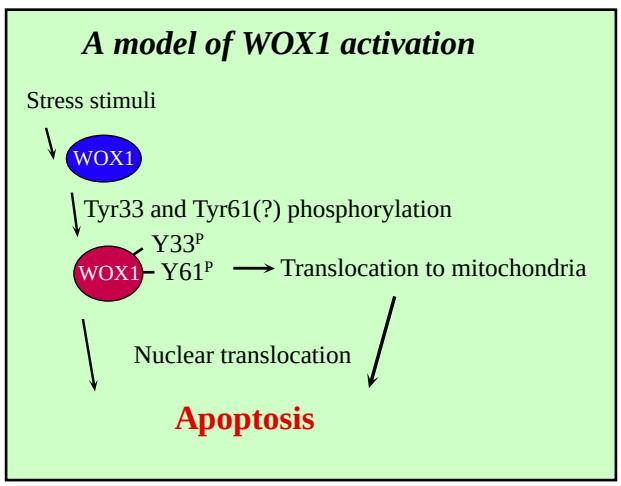

Fig. 3. Tyr33 phosphorylation and nuclear translocation are needed for WOX1-mediated apoptosis. Stress stimuli such as anisomycin and UV light induce phosphorylation of WOX1 at Tyr33 [16] and probably at Tyr61. This phosphorylated WOX1 can be considered as "activated" and is found mostly in the mitochondria (Chang *et al.*, unpublished), suggesting that WOX1 phosphorylation is essential for its translocation to the mitochondria from cytoplasm. Depending upon the strength of exogenous or endogenous stress or apoptotic signals, the activated WOX1 further translocates to the nuclei from either mitochondria or cytoplasm. Alteration of the nuclear localization signal (NLS) to prevent WOX1 nuclear translocation abrogates WOX1-induced apoptosis [15,16]. Thus, both Tyr33 (and/or Tyr61) phosphorylation and nuclear translocation are essential for WOX1 activation and its mediated apoptosis.

translocates to the nuclei from either mitochondria or cytoplasm. Alteration of the nuclear localization sequence to prevent WOX1 nuclear translocation abrogates WOX1-induced apoptosis [15,16]. Accordingly, both Tyr33 phosphorylation and nuclear translocation are essential for WOX1 activation and its mediated apoptosis (Fig. 3).

Another conserved phosphorylation site likely to be involved in WOX1 activation is Tyr61. Alteration of Tyr61 to a non-phosphorylating Gly or Arg abolishes p53 and WOX1 interaction, as determined in cytoplasmic Ras rescue-based yeast two-hybrid analysis (Chang *et al*., unpublished). Despite our discovery of the stress-induced WOX1 phosphorylation [16], the upstream protein tyrosine kinase(s) that phosphorylates WOX1 is unknown and remains to be isolated and characterized.

7. WOX1 physically interacts with the tumor suppressor p53 *in vivo*

We have determined that both p53 and WOX1 induce apoptosis in a synergistic manner [15]. A portion of WOX1 colocalizes with p53 in the cytosol [15]. Also, ectopically expressed p53 and WOX1 colocalize in the mitochondria in numerous cultured cells. However, in neonatal cardiomyocytes, the overexpressed p53 and WOX1 distribute evenly in the cytosol. TNF stimulates translocation of both proteins to the mitochondria (Chang *et al*., unpublished).

Apoptotic stimuli such as TNF, etoposide and UV light stimulate the synthesis and binding of endogenous Tyr33-phosphorylated WOX1 with activated p53 [15,16,26]. The proline-rich region of p53 physically interacts with the WW domains of WOX1, as determined by yeast two-hybrid analysis [15]. Also, phosphorylation of Ser46 in p53 and Tyr33 and 61 in the WW domains of WOX1 is essential for their binding interaction (Chang *et al*., unpublished). Alteration of these residues by site-directed mutagenesis disrupts the p53/WOX1 complex formation. Blocking of WOX1 expression or loss-of-function by antisense mRNA [15] or by a dominant negative WOX1 [16] abolishes p53 apoptosis, indicating that WOX1 is an essential partner of p53 in apoptosis. Most convincingly, siRNA-targeting WOX1 abolishes UV light-induced p53 phosphorylation at Ser15 (Chang *et al*., unpublished), further supporting the essential functional relationship between p53 and WOX1.

A similar example for this interaction is prolyl isomerase Pin1, which stabilizes phosphorylated or activated p53 (on Ser/Thr-Pro motifs) during DNA damage [28,29]. Pin1 possesses an N-terminal WW domain and a C-terminal isomerase domain. The WW domain in Pin1 binds to phosphorylated p53 on the Ser/Thr-Pro motifs and the poly-proline region. This type of binding is essentially identical to that of the WOX1 interaction with p53 (as described above). The isomerase activity of Pin1 further stabilizes its interaction with p53, thereby stimulating the DNA-binding activity and transactivation function of p53 [28,29]. Whether WOX1 stabilizes p53 and stimulates p53-mediated DNA binding and transactivation function remains to be established.

Recently, we determined that a portion of cytosolic IκBα, an inhibitor of NF-κB, physically interacts with p53 in cultured cells and organs [30]. This interaction allows IκBα to preserve a small amount of p53 in the cytosol from being degraded by the ubiquitin/proteasome system. In response to apoptotic stress, etoposide- and UV-mediated DNA damage, hypoxia, and transforming growth factor-β1 (TGF-β1)-mediated growth suppression, the p53/IκBα complex rapidly dissociates and p53 translocates to the nuclei [30]. Mapping by yeast two-hybrid analysis revealed that the PEST C terminus of IκBα physically interacts with the proline-rich region and the phosphorylation site, Ser46, in p53 [30]. There is no structural similarity between IκBα and WOX1 or Pin. However, these proteins interact with p53 to similar structural domains, indicating the importance of the poly-proline region and Ser46 in p53 for controlling growth regulation and apoptosis.

8. WOX1 and JNK1 interactions

JNK1 is a mitogen-activated protein kinase (MAPK) and is involved in cell growth, stress response, and apoptosis [31]. JNK1 possesses dual functional activities in cell growth regulation. Notably, transient activation of JNK1 protects cells against apoptosis, whereas persistent activation of JNK1 induces apoptosis [32]. The underlying mechanisms for the dual functions of JNK1 are unknown.

To promote cell survival, JNK1 may initiate cell cycle checkpoints and cell cycle progression [33]. Early activation of JNK1 is essential for protecting cells against TNF-mediated apoptosis [34,35]. Interestingly, the extracellular matrix-degrading enzyme hyaluronidase PH-20 enhances L929 cell growth and activates JNK1 and JNK2, thereby protecting these cells from staurosporine-mediated cell death [36]. JNK-regulated cell survival appears to be related with its phosphorylation of Bcl-2 [37].

While hyaluronidase PH-20 induces WOX1 expression [15] and JNK1 activation [36] in L929 cells, we examined whether the anti-apoptotic JNK1 interacts with the proapoptotic WOX1. We determined that ectopically expressed JNK1 inhibits WOX1-mediated apoptosis of L929 fibroblasts and other cell types [16]. Also, JNK1 blocks WOX1 prevention of cell cycle progression [16]. UV light activates JNK1 and p53 and induces WOX1 phosphorylation at Tyr33. The activated p53 physically interacts with the Tyr33-phosphorylated WOX1 and activated JNK1 [16]. Similarly, activated JNK1 binds to the phosphorylated WOX1, as determined by co-immunoprecipitation [16]. These observations suggest the formation of a heterotrimeric complex, p53/WOX1/JNK1, during stress response.

Alteration of Tyr33 to Arg33 in WOX1 abrogates its interaction with JNK1 and p53 [16], indicating that Tyr33 phosphorylation in WOX1 is essential for the binding interactions.

9. A potential role of WOX1 in congestive heart failure

Compared to other adult organs, WOX1 is abundant in the heart and the elastic membrane of blood vessel wall [15]. WOX1 protein is indeed overexpressed (by 0.7–3-fold increases) in patients with cardiac hypertrophy or cardiomegaly, compared to normal cardiac tissues (Chang *et al.*, unpublished). Whether WOX1 directly or indirectly induces hypertrophy of cardiac myocytes is unknown. Since TNF is involved in chronic and severe congestive heart failure (CHF) [38,39] and WOX1 is a downstream effector of the TNF apoptosis pathway [15], the over-expressed WOX1 protein is likely to enhance TNF-mediated apoptosis of cardiomyocytes that eventually lead to heart failure.

A proposed TNF signaling event that links to WOX1 activation in adult cardiomyocytes is shown (Fig. 4). TNF induces WOX1 activation via Tyr33 phosphorylation, followed by translocating to the mitochondria and nuclei. However, the upstream kinase(s) that phosphorylates WOX1 is unknown. The activated WOX1 interacts with (1) TRAF2, (2) TRADD, (3) p53 [15], and (4) p53 and JNK1 [16]. Hypertrophic and apoptotic responses in TNF-treated adult cardiomyocytes are likely due to the formation and nuclear translocation of the p53/WOX1/JNK1 complex. A p53/WOX1 complex is also generated and migrates to the mitochondria to alter the mitochondrial function. Also, apoptosis can occur as a consequence of WOX1 interaction with TRADD and TRAF2.

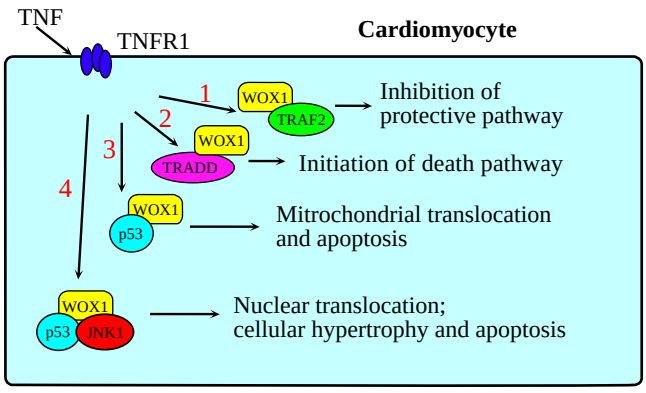

Fig. 4. A TNF signaling event that links to WOX1 activation in cardiomyocytes. TNF induces WOX1 phosphorylation at Tyr33 via an unknown kinase. The activated WOX1 binds (1) TRAF2, (2) TRADD, (3) p53 [15], and (4) p53 and JNK1 [16]. Hypertrophic response in TNF-treated cardiomyocytes is likely due to the formation and nuclear translocation of a hetero-trimeric p53/WOX1/JNK1 complex. Also, apoptosis can occur as a consequence of WOX1 interaction with p53, JNK1, TRADD, and TRAF2.

10. Other redox proteins interact with p53

A further example that oxidoreductases constitute important components in stress responses is another mammalian oxidoreductase, NADH quinone oxidoreductase 1 (NQO1). NQO1 has been shown to stabilize p53 by inhibiting its degradation in a distinct pathway independent of ubiquitination [40]. How NQO1 interacts with p53 is unknown. Furthermore, a bacterial redox protein, azurin, has been shown to bind p53 and induce apoptosis in melanoma cells [41]. Interestingly, azurin enters cancer cells, increases p53 levels, and binds p53. The p53/azurin complex localizes in the cytosol and nuclei. The complex is also present in the mitochondria and causes an increased Bax level that allows cytochrome *c* release in this organelle [41]. Although azurin stabilizes p53, the interacting domains in these proteins are not identified.

11. Summary: WOX1 proapoptotic vs. anti-apoptotic?

Taken together, our studies have established that WOX1 participates in multiple signaling pathways. WOX1 interacts with TRADD and TRAF2 of the TNF signaling pathway (Chang *et al.*, unpublished), JNK1 of the cellular stress pathway [16], and IκBα/p53 for apoptotic response [30,32]. Further likely important interactions are that (1) WOX1 metabolizes steroid hormones, and (2) WOX1 interacts with other WOX proteins. WOX1 possesses the conserved catalytic tetrad N-S-Y-K at the SDR/ADH domain. This allows WOX1 binding with steroid hormones. Whether the N-terminal WW domains affect the binding interactions remains to be determined. Additionally, our preliminary study showed that WOX1 appears to interact with WOX3 (or v6) in breast and prostate cancer cells. WOX3 is over-expressed in these cancer cells. Since WOX1 is a candidate tumor suppressor, the WOX1 activity may be neutralized by WOX3, thereby promoting tumor growth.

AIF is a mitochondrial oxidoreductase [42]. Once released from the mitochondria, AIF induces caspase-independent apoptosis at the nuclear level. Nonetheless, the redox activity of AIF is essential for scavenging free radicals in the mitochondria [43,44], indicating that AIF is also a pro-survival factor. Whether WOX1 is capable of scavenging free radicals is unknown. However, WOX1 plays a critical role in the developing nervous system. High levels of WOX1 expression are observed in the neural crest-derived structures such as cranial and spinal ganglia, skin pigment cells and mesenchyme in the head, indicating a potential role of WOX1 in the neuronal differentiation and cellular maturation (Chen *et al.*, submitted). Here, we present a large body of information on the functional role of WOX1. However, open questions and future lines of research as outlined in this review remain and are pursued in our laboratories.

Acknowledgments

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