Thioredoxin Interacting Protein: Redox Dependent and Independent Regulatory Mechanisms

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Abstract

Significance: The thioredoxin-interacting protein (TXNIP, also termed VDUP1 for vitamin D upregulated protein or TBP2 for thioredoxin-binding protein) was originally discovered by virtue of its strong regulation by vitamin D. Recently, TXNIP has been found to regulate the cellular reduction-oxidation (redox) state by binding to and inhibiting thioredoxin (TRX) in a redox-dependent fashion. **Recent Advances:** Studies of the Hcb-19 mouse, TXNIP nonsense mutated mouse, demonstrate redox-mediated roles in lipid and glucose metabolism, cardiac function, inflammation, and carcinogenesis. Exciting recent data indicate important roles for TXNIP in redox independent signaling. Specifically, sequence analysis revealed that TXNIP is a member of the classical visual/ β -arrestin superfamily, and is one of the six members of the arrestin domain-containing (ARRDC, or α -arrestin) family. **Critical Issues:** Although the function of α -arrestins is not well known, recent studies suggest roles in endocytosis and protein ubiquitination through PPxY motifs in their C-terminal tails. Importantly, the ability of TXNIP to inhibit glucose uptake was found to be independent of TRX binding. Further investigation showed that several metabolic functions of TXNIP were due to the arrestin domains, thus further supporting the importance of redox independent functions of TXNIP. **Future Directions:** Since TXNIP transcription and protein stability are highly regulated by multiple tissue-specific stimuli, it appears that TXNIP should be a good therapeutic target for metabolic diseases. *Antioxid. Redox Signal.* 16, 587–596.

Introduction

OXIDATIVE STRESS IS CAUSED by a shift in balance from the normal reduced cellular environment to a state of excessive reactive oxygen species (ROS) generation. This may occur by increases in the generation of ROS and/or by decreases in the mechanisms that reduce ROS: These include antioxidant molecules such as glutathione, thioredoxin 1 and 2 (TRX1 and TRX2), and glutaredoxin (72). In this article, we focus on thioredoxin-interacting protein (TXNIP) because of recent discoveries that TXNIP can inhibit TRX1 and TRX2 (58, 76) (termed TRX unless specifically mentioned), thereby playing a role in reduction-oxidation (redox) signaling, but also it has a role in cellular signaling events because of its function as a scaffold protein.

TRXs are a family of proteins with a conserved catalytic site (Cys-Gly-Pro-Cys) that undergoes reversible oxidation to cysteine disulfide (TRX-S₂) through the transfer of reducing equivalents from the catalytic site cysteine residues (Cys) to a disulfide substrate (protein-S₂). The oxidized TRX is then reduced back to the Cys form (TRX-(SH)₂) via the action of NADPH-dependent flavoprotein TRX reductases (35).

TRX is important for the regulation of multiple biological processes in a cell-compartment specific fashion. These include regulation of cell growth and angiogenesis in the nucleus and regulation of redox state, inhibition of apoptosis signal-regulating kinase 1 (ASK1) in the cytosol, and regulation of plasma membrane receptor signaling (21, 47, 59, 67, 71, 72, 75).

TXNIP was originally identified in HL-60 leukemia cells treated with 1,25-dihydroxyvitamin D₃ and was termed vitamin D up-regulated protein 1 (VDUP1) (15). Thereafter, Nishiyama *et al.* isolated TXNIP using a yeast two-hybrid system as a TRX1-binding protein and was termed thioredoxin-binding protein 2 (TBP2) (44). There are two ways that TXNIP can inhibit the function and action of TRX: First, TXNIP binds TRX and acts as a competitive inhibitor to remove TRX from proteins whose function is inhibited by the steric effect of TRX1 binding, such as ASK1 (76). Second, increases in TXNIP expression by factors such as disturbed flow and high glucose result in reduced TRX reductase activity (69, 76, 81, 84). The increase in TXNIP-TRX complexes will result in more oxidized proteins on exposure to oxidative stress. TXNIP inhibits TRX reductase activity by forming a mixed

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FIG. 1. Model for TRX regulation by TXNIP. TRX regulates cellular redox state by reducing oxidized cysteine residues through TRX catalytic core, resulting in oxidized form of TRX and reduced residues on target proteins. TXNIP, via Cys247, forms a disulfide bond with TRX catalytic site Cys32 to inhibit TRX activity and function. TRX, thioredoxin; TXNIP, thioredoxin-interacting protein; redox, reduction-oxidation; Cys, cysteine residue.

disulfide bond between the TRX active site cysteine 32 and TXNIP cysteine 247, as detailed in Figure 1 (52). This interaction is redox dependent (Table 1) and only exists between the oxidized form of TXNIP and the reduced form of TRX (52). It is important to note that the literature describing TRX activity should be read carefully with regard to TXNIP, because the two most popular methods yield different results. In one method that usually involves reduction of insulin, total TRX activity is measured after a reducing agent such as dithiothreitol is added to reduce any Cys, thereby disrupting TRX-TXNIP complexes (24). This method yields total TRX activity and does not reflect the effect of TXNIP binding. In the second method, no reducing agent is used and will measure the effect of TRX activity when binding of the other proteins is intact, including potential activators such as TRX-reductase and inhibitors such as TXNIP.

TABLE 1. SUMMARY OF KNOWN THIOREDOXIN-INTERACTING PROTEIN FUNCTIONS AND EFFECTS BASED ON *IN VITRO* EXPERIMENTS

<i>Redox-dependent</i>	<i>Redox-independent</i>
Inhibition of TRX	Induction of TXNIP expression by glucose
Induction of oxidative stress	Regulation of PPARs and insulin secretion
Promotion of inflammation	Regulates liver-glucose homeostasis
Regulation of mitochondria function	Induction of apoptosis, Cas-3 cleavage
Down-regulation of TXNIP by nitric oxide	Involvement in cancer and cell growth
Mechanosensor	Suppression of proliferation
Role in cardiac function and hypertrophy	Interaction with HDACs
Regulation of cardiomyocytes viability	Regulation of HIF1- α
Regulation of angiogenesis	Promotes glucocorticoid-induced apoptosis

TRX, thioredoxin; TXNIP, thioredoxin-interacting protein; HIF1- α , hypoxia inducible factor 1- α ; PPARs, peroxisome proliferator activated receptors; HDAC, histone deacetylase.

TABLE 2. SUMMARY OF KNOWN *IN VIVO* THIOREDOXIN-INTERACTING PROTEIN FUNCTIONS

<i>Organ/Function</i>	<i>TXNIP in vivo effects</i>
Liver	Increased fatty acid synthesis, cholesterol accumulation TXNIP down-regulation protects from liver I-R injury
Pancreas	β -cells apoptosis, development of diabetes Regulation of insulin secretion and response Regulation of peripheral glucose metabolism
Cell Growth/Cancer	Development of hepatocellular carcinoma Involved in renal carcinogenesis Role in lung development
Inflammation	NLRP3 inflammasome activation Regulation of hepatic natural killer cells
Cardiac	Regulation of cardiac homeostasis and remodeling Mechanosensor and inducer of cardiac myocytes apoptosis
Vasculature	Induction of EC inflammation

I-R, ischemia-reperfusion; EC, endothelial cell; NLRP3, NLR protein 3.

Based on it belonging to the α -arrestin scaffold family (3), TXNIP is suggested to exert redox independent functions, as detailed in Tables 1 and 2. Specifically, TXNIP was shown to function as a cell growth and transcription repressor (27), metabolic regulator (41, 51, 54, 65, 81), modulator of inflammatory response (70, 83), cardiac function (69, 73, 78, 79), and vascular cells signaling and apoptosis (53, 60, 72, 76).

This article will contextualize the information regarding TXNIP redox-dependent and -independent mechanisms based on a structure function analysis as well as recent findings from tissue specific TXNIP Knock-out (KO) mouse, TXNIP nonsense mutated mouse (HcB-19 strain), and *in vitro* studies (Tables 1 and 2).

TXNIP Domain Structure Analysis

To understand the potential scaffold function of TXNIP, we combined three analyses of protein structure and function. First, TXNIP was recently shown to possess homology to β -arrestins and to be one of at least six members of a novel family of proteins termed α -arrestin or arrestin domain containing proteins (ARRDC) (3) as detailed in Figure 2. β -arrestin proteins associate with the plasma membrane where they facilitate the endocytosis of G-protein coupled receptors and attenuate signaling (68). The archetypal β -arrestins are composed of two major structural domains. The NH2 domain contains sites for protein-protein interaction; in particular, two SH3-binding domains (PxxP), shown to bind Src (36) and a mitogen activated protein kinase kinase kinase (MAPKKK) ASK1 binding domain (40). In contrast, the COOH domain contains motifs that mediate the interaction with the Mdm2 E3 ubiquitin ligase and importantly contains two sites that interact with adaptin and clathrin heavy chain (32), which together regulate endocytosis of membrane-associated receptors.

Second, we used the sequence comparison among the α -arrestins identified by Alvarez (3) to identify seven conserved

FIG. 2. Sequence alignment of TXNIP and representatives of each of the major arrestins families, demonstrating several conserved domains. Within the described domains can be noticed domains that shared within all the arrestin super family (yellow boxes), TXNIP and α -arrestins (ARRDC 2–4) in blue boxes, or only within the β -arrestins (green and red boxes). Important to notice, SH3 and PPxY that are known to mediate protein-protein interactions are conserved within the arrestin super family. ARRDC 2–4 present high identity (>33%) and similarity (>50%) to TXNIP, whereas ARRDC 1 and 5 have lower values (20% and 35% respectively). ARRDC, arrestin domain containing protein.

domains that TXNIP shares with the arrestins, which includes SH3 and PPxY domains (Fig. 2).

Third, to identify which critical residues and domains would be surface exposed to facilitate protein-protein interactions, we performed a three-dimensional (3D) analysis of TXNIP structure, as detailed in Figure 3 (LOOPP@BioHPC

FIG. 3. TXNIP 3D structure using the LOOPP@BioHPC Analysis offered by the Computational Biology Service Unit from Cornell University. This model demonstrates important surface residues and domains that are proposed to mediate redox-independent TXNIP functions and protein-protein interactions, such as SH3 (residues 106–109 and 132–135), ITIM (Tyr279), and CRM1 (Leu294). In addition, the Cys247, which mediates interaction with TRX, can be found on the surface of the protein. The 3D structure presented here demonstrates a “W”-like shape, which contains potential domains that can act as docking sites for target proteins. The LOOPP@BioHPC analysis is a fold recognition program. This program generates 3D map of specific protein based on atomic coordinates that accounts for sequence alignment, secondary structure, and exposed surface area predictions. ITIM, immunoreceptor tyrosine-based inhibition motif; CRM1, chromosome maintenance region 1; 3D, three-dimensional. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Analysis offered by the Computational Biology Service Unit from Cornell University).

Combining the sequence homology and the 3D analysis, we developed a structure-function cartoon of TXNIP (Fig. 4) demonstrating domains with known or proposed roles in specific signaling pathways. The TXNIP NH2 domain contains two SH3-binding domains, and the COOH domain contains two PPxY motifs and three SH3 domains. We propose that the SH3 domains mediate TXNIP scaffold properties in distinct cellular locations (nuclear, cytosol, and plasma membrane). Further, it is important to notice an immunoreceptor tyrosine-based inhibition motif domain (Tyr279), which facilitates the interaction with phosphotyrosine phosphatases such as SHP-2. Those phosphatases are known to regulate important cellular responses, including activation of plasma-membrane-associated receptors (4). We also included the PPxY motif, because published data show that the E3-ubiquitin ligase Itch mediates TXNIP ubiquitination and degradation specifically through those domains (Fig. 4). Further, yeast members of the α -arrestins use the PPxY motif to promote budding and cargo transport to the plasma membrane (57, 82). In addition, chromosome maintenance region 1 (CRM1), specifically leucine 294, binds the nuclear transcription factor hypoxia inducible factor 1- α (HIF1- α) and the ubiquitin ligase von Hippel-Lindau protein (pVHL). The formation of TXNIP-pVHL-HIF1- α complex results in nuclear export and degradation of HIF1- α in the cytoplasm and inhibition of HIF1- α function, including cell invasion (64). The multiple domains observed within TXNIP sequence, as just described above, suggest that TXNIP plays an important role in multiple cellular functions. Finally, the redox sensitive functions of TXNIP are largely mediated by Cysteine 247 to form an intramolecular mixed disulfide bond with TRX catalytic site (Fig. 1) (52).

Phenotype of TXNIP KO Mouse

Early studies that investigated the effect of *in vivo* nonsense mutated TXNIP utilized the HcB-19 mouse strain. This strain develops hyperlipidemia due to an elevation in fatty acid

FIG. 4. Model for TXNIP structure-function. Based on TXNIP sequence and structure analysis, we generated a map describing known (red arrows) and proposed (black arrows) functions of TXNIP in several cellular processes: (A) Inflammation—induction of oxidative stress and inflammation *via* the binding and inhibition of TRX. (B) Mitochondria function—inhibition of TRX2 alters mitochondria function and promotes oxidative stress. (C) Plasma membrane TKRs regulation—TXNIP is proposed to regulate TKRs activity *via* the formation of protein complex including SHP2 phosphatase. (D) Nuclear functions—HIF1- α translocation from the nucleus to the cytosol

for proteasomal degradation. In addition, TXNIP function as a transcriptional repressor and was also shown to interact with nuclear HDACs. (E) TXNIP is proposed to regulate plasma membrane integrin-mediated signaling *via* formation of protein complex including Src and PI3K. TKRs, tyrosine kinase receptors; HIF1- α , hypoxia inducible factor 1- α ; HDACs, histone deacetylases.

synthesis and cholesterol accumulation (20). The causative genetic defect in this strain was identified as a nonsense mutation in the TXNIP gene that resulted in >90% reduction of TXNIP expression (5). Due to the alterations in liver function identified in the HcB-19 mouse, initial studies focused on the role of TXNIP as a metabolic regulator. However, further analyses of phenotypes exhibited by the TXNIP KO mouse demonstrated multiple roles of TXNIP in both redox-dependent and independent functions. As detailed in Table 2, TXNIP has been demonstrated to play a crucial role in several pathological conditions, including metabolic syndrome (5, 8, 13, 26, 38), inflammation (49, 83), cancer (7, 22, 31, 43, 74), cardiac (78, 79, 81), and vascular function (76).

TXNIP in Glucose Signaling and Metabolic Syndrome

Metabolic abnormalities in the HcB-19 mouse have been well characterized. Key features include induction of TXNIP by glucose, alterations in insulin secretion by pancreatic β -cells, and switch from gluconeogenesis to glyceroneogenesis and lipogenesis (10, 12, 26, 41, 50).

There are multiple pathways by which extracellular glucose regulates TXNIP expression (84). The first is mediated by the transcription factor MondoA and Max-like protein X (MLX) (54, 81). The underlying mechanism to explain TXNIP upregulation is related to increased glycolytic intermediates, which are inducers of MondoA:MLX binding to carbohydrate response element found within the TXNIP promoter. This interaction increases TXNIP promoter activity and enhances TXNIP expression (41, 65, 80). Recently, Yu *et al.* showed that mitochondrial oxidation-phosphorylation inhibitors lead to increase in glycolytic flux and decreased levels of glycolytic intermediates, which are important for the function of MLX and MondoA and TXNIP expression (80). More recently, Chai *et al.* also reported that under hypoxia, TXNIP expression is reduced *via* the suppression of the MondoA:MLX cascade, which is mediated

via depletion of glycolytic intermediates (9). Second, Li *et al.* showed that glucose enhanced TXNIP expression *via* p38 MAPK and the transcription factor FOXO1, which lead to impaired TRX activity and induction of apoptosis (34, 41). Third, TXNIP regulation of glucose homeostasis was also linked to peroxisome proliferator-activated receptors (48, 55). Fourth, using Genomatix software, Qi *et al.* described seven Kruppel-like factor 6 (KLF6) binding sites in TXNIP promoter, thus suggesting additional mechanisms for the regulation of TXNIP expression (55). Finally, in cancer cells, it appears that TXNIP is regulated by class II histone deacetylases (HDACs), such as HDAC10 (6, 33).

A very important role for TXNIP has been proposed in the pathogenesis of diabetes due to its induction by hyperglycemia, as well as regulation of insulin sensitivity, glucose uptake, glucose stimulated insulin secretion, and beta cell apoptosis. In an elegant human study, Lee and colleagues showed that TXNIP expression in healthy individuals was elevated in the muscle of prediabetics and patients with diabetes. Further, TXNIP expression correlated inversely with total body measures of glucose uptake (50). In two mice models of adipose-mediated hyperglycemia and glucose-intolerance, TXNIP depletion improved insulin sensitivity and lowered glucose. Yoshihara *et al.* showed in obese model mice that TXNIP deficiency enhanced insulin sensitivity with activated insulin receptor substrate-1/protein kinase B (Akt) signaling (77). Chutkow *et al.* showed that fat-fed TXNIP KO mice were markedly more insulin sensitive than controls, and augmented glucose transport was identified in both adipose and skeletal muscle (16). Further support for the Akt pathway was reported by Hui *et al.*, who showed regulation of the phosphatase and tensin homolog (PTEN)-Akt signaling cascade by TXNIP (25).

The data to support the role of TXNIP redox-dependent pathways in beta cell apoptosis is strong. TXNIP overexpression in β -cells leads to enhanced apoptosis and impaired glucose and lipid metabolism (14). Conversely, a

decrease in TXNIP restored glucose regulated insulin secretion, beta cell apoptosis, and reduced ROS generation (56). TXNIP degradation was also correlated with reduced caspase-3 cleavage and reduced apoptosis of β -cells (63). Further, in β -cells, Shao *et al.* showed that proteasome-mediated TXNIP degradation inhibited high-glucose-mediated β -cell apoptosis, by ubiquitination and targeting of TXNIP to proteasomal degradation.

One of the phenotypes exhibited by the HcB-19 mouse is altered liver function, which includes increased lipogenesis and glyceroneogenesis (26). Insulin levels of the HcB-19 are elevated compared with wild-type (WT) mouse with associated increased hepatic mRNA levels of the transcription factor sterol regulatory element-binding protein-1c, which promotes lipogenic conditions. The transition to a lipogenic and glyceroneogenic metabolic state is related to the ability of TXNIP to affect the cellular redox state (26). Donnelly *et al.* reported increased hepatic triacylglycerol stores, liver weight, and total cholesterol levels in the HcB-19 mouse, which was related to increased lipogenesis and fatty acid reesterification, thus demonstrating the critical role that TXNIP plays in liver function (20). Further, Chutkow *et al.* used a hepatocyte-specific TXNIP-KO mouse to demonstrate that TXNIP is important for liver-glucose homeostasis. TXNIP null hepatocytes demonstrated reduced glucose production that was rescued by reintroducing TXNIP-WT. Interestingly, overexpression of TXNIP mutant C247S failed to restore liver glucose production, although TRX activity was not affected (18). These data suggest that the TXNIP-TRX complex has unique signaling properties independent of changes in TRX activity.

TXNIP in Cell Growth and Cancer

TXNIP plays an important role in cell growth and cancer acting as a tumor suppressor as shown by dramatically reduced expression in multiple tumors including renal, breast, lung, gastric, colon, and hepatocellular carcinoma (7, 22, 29, 31, 33, 66). This observation is likely due to both the pro-apoptotic and anti-proliferative properties of TXNIP. Specifically, TXNIP promotes apoptosis by facilitating activation of ASK1 by relieving the constitutive inhibition of ASK1 due to pre-bound TRX (39). In addition, TXNIP expression was strongly induced by dexamethasone and was required for glucocorticoid-induced apoptosis of thymocytes (70). To induce cell growth arrest, TXNIP is required to localize to the nucleus. Nishinaka *et al.* demonstrated that TXNIP growth-suppressive activity is dependent on its ability to interact with importin α , *via* non-classical nuclear localization signal (42).

Nuclear TXNIP regulates cell proliferation by multiple mechanisms: (i) TXNIP increases the stability of the cyclin-dependent kinase (CDK) inhibitor p27(kip1), *via* inhibition of jun activating binding protein 1 (27, 74). Possible mechanism to explain this observation includes redox-sensitive interaction of p300 and forkhead box protein O4 (FoxO4) that is regulated by TRX and TXNIP. Over-expression of TXNIP inhibits TRX activity that results in increased p300-FoxO4 complexes resulting in increased p27(kip1) and G1 cell growth arrest (19). (ii) Nishinaka *et al.* showed that over expression of TXNIP leads to increased levels of the CDK inhibitor p16, reduced retinoblastoma phosphorylation, and subsequent G1 cell growth arrest (43). In conclusion, TXNIP is a novel tumor suppressor *via* its effects to regulate both cell growth and death.

TXNIP in Inflammation

It is clear that TXNIP plays a critical role in inflammation by virtue of its ability to dissociate TRX from ASK1, thus enabling activation of the ASK1-c-jun-N-terminal kinase (JNK) pathway in response to multiple stimuli. In endothelial cells (EC), activation of this pathway by tumor necrosis factor (TNF) was shown to be responsible for the increase in vascular cell adhesion molecule 1 expression (76). In a different report, ceramide, a key mediator in the TNF and sphingosine 1 inflammatory pathways, was also shown to stimulate JNK activation, in part by upregulating TXNIP expression in mouse T-hybridoma 10I cells and human Jurkat T cells. (10).

A recent report described TXNIP as a link between oxidative stress and inflammasome activation contributing to the innate immunity response (83). The NLR protein 3 (NLRP3) inflammasome is responsible for the production of mature interleukin-1 β (IL-1 β) in response to cellular stress, including elevated ROS. Specifically, ROS promoted the dissociation of TXNIP from TRX1 and enhanced interaction between TXNIP and NLRP3 inflammasome to promote IL-1 β production. These results suggest a competition between TRX and NLRP3 for TXNIP binding. Interestingly, the TXNIP KO mouse is similar to the NLRP3 KO mouse in exhibiting improved glucose tolerance and insulin sensitivity (83).

TXNIP Role in Cardiac Function

TXNIP plays an important role in the response of the heart to pressure overload and ischemia-reperfusion (I-R). The first publication that demonstrated a role for cardiac TXNIP showed that TXNIP was the most highly down-regulated gene in response to stretch of cardiomyocytes (69). Further, TXNIP was induced in a ROS-dependent fashion and positively correlated with cardiomyocytes apoptosis (69). Using an inducible cardiomyocytes-specific TXNIP-KO mouse, the Lee group showed that in response to pressure overload TXNIP-KO hearts had less cardiac hypertrophy and better left ventricular function through 4 weeks of pressure overload (78). However, these beneficial effects were lost by 8 weeks of pressure overload, and maladaptive remodeling was observed. The effects of TXNIP deletion were not due to increased TRX activity or alterations in ROS but due to changes in metabolism. Specifically, TXNIP-KO hearts exhibited greater myocardial glucose uptake (78). More recently, the Sadoshima group found that TXNIP functioned as a scaffold to shuttle TRX1 into the nucleus where it regulated the transcriptional activity of HDAC4, suppressing hypertrophic gene expression (2). These studies demonstrate the complex roles of TXNIP that involve both redox dependent and independent pathways.

In I-R models, TXNIP expression correlates with TRX inhibition, apoptosis, and worse tissue injury (73). After I-R injury, TXNIP is induced, and promotes oxidative stress by inhibiting TRX. Administration of trans-resveratrol (both anti-oxidant and HDACs inhibitor) reduced TXNIP expression, which was accompanied by increased TRX activity and subsequently prevented I-R induced injury (45). Interestingly, cardiac TXNIP expression can be reduced by calcium channel blockers, resulting in reduced caspase-3 cleavage and apoptosis (11), although the exact mechanism is still unknown.

TXNIP Function in Vasculature

TXNIP plays an important role in vascular smooth muscle cells (VSMC) and EC of the vasculature. In VSMC, it appears that TXNIP plays a prominent role in regulating TRX activity and, hence, VSMC redox state. Over-expression of TXNIP in VSMC abolished platelet-derived growth-factor-induced TRX activity and DNA synthesis (60). This result suggests that TXNIP has antiproliferative effects in VSMC by suppressing TRX activity. Further, Schulze *et al.* demonstrated that under hyperglycemic conditions, TXNIP was induced and contributed to increased VSMC oxidative stress due to TRX inhibition (62). Finally, it appears that TXNIP represents an important mediator for EC-VSMC communication. Specifically, increasing nitric oxide down-regulated TXNIP mRNA expression, thus suggesting that normal EC function and generation of nitric oxide represents a chronic mechanism to maintain VSMC in reduced redox state (61).

TXNIP is a critical regulator of pathologic EC function as shown by multiple effects such as inflammation, apoptosis, oxidative stress, and inhibition of endothelial nitric oxide synthase (eNOS) function. In normal EC, there is very little TXNIP expressed but it can be highly induced by several pathologic stimuli. Initially, it was shown that TXNIP was induced by high glucose through the MondoA:MLX pathway. Subsequently, TXNIP expression was found to be suppressed by physiologic steady laminar flow, and induced under conditions of low or disturbed flow (71, 76). The beneficial effects of steady flow appear to be related to the induction of the growth inhibitor p21 that suppresses TXNIP expression in EC, thus resulting in reduced cell adhesion molecules expression (46). These data are supported by the evidence that p21 regulates TRX secretion and angiogenesis *via* transcriptional repression of TXNIP (30). In agreement with these data, TXNIP induces inflammation in retinal capillary EC, under diabetic conditions, by activation of inflammatory genes such as Cox2 and ICAM-1 (53). These data demonstrate TXNIP as a mechanosensitive sensor to regulate EC function and inflammation.

Further, TXNIP has been shown to play an important role in EC migration and angiogenesis. Recently, we reported that in response to low oxidative stress, TXNIP mediates cell survival and migration in a TRX1-dependent fashion. Cell survival and migration are dependent on the formation of TXNIP-TRX complex and a translocation to the plasma membrane to regulate vascular endothelial growth factor receptor 2 (VEGFR2) signaling (71). In addition, there are substantial data to support a key role for TXNIP in angiogenesis *via* regulation of the HIF1- α -VEGF pathway (23). Farrel *et al.* reported that TXNIP inhibited VEGF signaling, by reducing the stability of HIF1- α protein in a TRX-independent fashion (23). The authors propose that TXNIP regulates HIF1- α function by affecting its stability and degradation. This was supported by the demonstration that TXNIP mediates HIF1- α translocation from the nucleus to the cytoplasm and targets HIF1- α to degradation (64).

Critical Issues and Future Directions

In this article, we have highlighted the redox independent functions of TXNIP, which may have equal importance compared with the redox dependent functions (Table 1 and 2). Redox dependent functions of TXNIP are related to its ability to bind TRX and regulate TRX activity (Fig. 5). The redox independent functions we have discussed are first that TXNIP functions as an arrestin (independent of binding to TRX) in specific sub-cellular compartments, and second that TXNIP-TRX complexes act as signaling mediators. It is important to mention that TXNIP-TRX interaction is redox sensitive. It is suggested that after the formation of the TXNIP-TRX complex, redox-independent events may occur to regulate cellular responses. Further, a relative small amount of TRX bound to TXNIP can have major signaling effects in a specific sub-cellular location (71).

As described in Figure 5, it appears that TXNIP expression is regulated by multiple mechanisms, which can provide explanations for TXNIP ability to contribute to signaling cascades. First, TXNIP is highly induced by glucose in a

FIG. 5. Summary of TXNIP regulation and function. Several factors are known to regulate TXNIP expression, such as glucose, blood flow, HDACs, nitric oxide, and more. As a result of changes in TXNIP expression, three main effects can be noticed: (i) TRX inhibition, (ii) TXNIP-TRX complexes formation and functions, and (iii) TXNIP ability to function as a scaffold. Alterations in those three mechanisms are proposed to be the underlying mechanism involved in TXNIP-regulated cellular processes.

mechanism that involves both MondoA: MLX and p38-FOXO1. Second, TXNIP is regulated by the activity of HDACs, KLFs, and peroxisome proliferator activated receptors in mechanisms that still need to be investigated. Third, TXNIP expression is regulated by blood flow properties. Under steady laminar flow, TXNIP expression is strongly reduced, by a mechanism that depends on eNOS activity and the generation of NO (61). In contrast, under low and/or disturbed blood flow conditions, TXNIP is significantly induced (71, 76). A recent publication suggests Nrf2, an important transcription factor known to regulate anti-oxidant response, as a regulator of TXNIP expression (1). The authors showed a Nrf2-mediated increase in TXNIP expression in response to cigarette smoke. To strengthen those findings, we analyzed the TXNIP gene promoter for Nrf2 binding motif, based on a sequence described by Malhotra *et al.* (37). We identified an Nrf2 binding motif located 1689 base pairs upstream of TXNIP gene. This suggests that Nrf2 is a good candidate to regulate TXNIP expression.

Alterations in TXNIP expression regulate its ability to affect several biological processes, as described in Figure 5. First, TXNIP plays a crucial role in development of inflammatory processes: (i) release of ASK1 from TRX to up-regulate pro-inflammatory gene expression; and (ii) regulation of NLRP3 inflammasome activation and IL1 β maturation. NLRP3 regulation by TXNIP, as shown by Zhou *et al.*, requires dissociation of TXNIP-TRX complex in a redox-dependent fashion (83). The underlying mechanisms to explain such a TXNIP-TRX interaction remain to be further explained. Second, TXNIP plays an important role as a metabolic regulator. The HcB-19 mouse that has hyperlipidemia, altered liver function, altered insulin secretion and glucose uptake, and mitochondria dysfunction best exemplifies this. Third, TXNIP can promote cell apoptosis *via* redox dependent inhibition of TRX that results in excessive generation of ROS, and redox independent activation of ASK1 and caspases. Fourth, TXNIP is an important transcription repressor, in particular genes that regulate cell growth such as CDK inhibitor p27(kip1) and p16.

Functionally, based on the biology of TXNIP just described, it is not unexpected that TXNIP plays an important role in several pathologic conditions (Fig. 5). To date, TXNIP was shown to be involved in diabetes, hyperlipidemia, cancer development, cardiac remodeling and hypertrophy, EC inflammation and dysfunction, and cell death.

Our understanding of the mechanisms by which TXNIP contributes to the described pathologies is limited. For example, TXNIP is known to localize to specific sub-cellular compartments to regulate several signaling cascades, but the mechanism underlying shuttling and localization is not known. In addition, it is not clear what are the cellular events that require a TXNIP-TRX complex or TRX-free TXNIP and how this interaction is regulated. The hypothesis that TXNIP-TRX interaction is not limited to TRX function inhibition is being supported by recent publications. Chutkow and Lee recently reported that TRX can enhance TXNIP stability by direct interaction *via* Cys247 and regulating adipogenesis (17). In addition, we showed TXNIP-TRX complex mediated activation of plasma membrane signaling to promote cell survival and migration (71). The TXNIP-TRX effects can be related to TXNIP as a scaffold protein as an α -arrestin protein. The understanding of this function is still limited, as only a few reports describe it as a scaffold to interact with target proteins

other than TRX (3, 31, 64, 71). In addition, it can be hypothesized that TRX acts as a regulator of TXNIP function *via* induction of structural alterations, regulation of redox-dependent protein interactions, and/or changes in TXNIP sub-cellular localization and function. In addition, although it was shown that TXNIP shuttles to the mitochondria resulting in TRX2 inhibition (58), the differential effects of TXNIP on cytoplasmic TRX1 *versus* mitochondrial TRX2 remain to be investigated. Further, TXNIP role as a mechanosensor of EC is partially understood at the expression level but poorly understood at the functional level (76). In addition, recent evidence demonstrates a new role for TXNIP as a regulator of mammalian target of rapamycin (mTOR) cascade signaling *via* increasing the stability of Redd-1 by blocking its degradation (28). Finally, although TXNIP is tightly regulated, its posttranslational modifications have not been described.

We suggest that TXNIP is a good therapeutic target because of its multiple roles, cells, and tissue-specific mechanisms that regulate TXNIP expression and function as well as the multiple pathologic conditions which TXNIP may regulate.

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Abbreviations Used

3D = three-dimensional
Akt = protein kinase B
ARRDC = arrestin domain containing proteins
ASK1 = apoptosis signal-regulating kinase 1
CDK = cyclin-dependent kinase
CRM1 = chromosome maintenance region 1
Cys = cysteine residue
EC = endothelial cell
eNOS = endothelial nitric oxide synthase
FoxO4 = forkhead box protein O4
HDAC = histone deacetylase
HIF1- α = hypoxia inducible factor 1- α
IL-1 β = interleukin-1 β
I-R = ischemia-reperfusion
ITIM = immunoreceptor tyrosine-based inhibition motif
JNK = c-jun-N-terminal kinase
KLF = Kruppel-like factor
KO = knock-out
NLRP3 = NLR protein 3
MAPKKK = mitogen activated protein kinase kinase kinase
MLX = Max-like protein X
pVHL = von Hippel-Lindau protein
redox = reduction-oxidation
ROS = reactive oxygen species
TBP2 = thioredoxin-binding protein 2
TKRs = tyrosine kinase receptors
TRX = thioredoxin
TXNIP = thioredoxin-interacting protein
VDUP1 = vitamin D up-regulated protein 1
VEGFR2 = vascular endothelial growth factor receptor 2
VSMC = vascular smooth muscle cell
WT = wild type