



## FORUM REVIEW ARTICLE

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# Physiological and Pathophysiological Roles of Thioredoxin Interacting Protein: A Perspective on Redox Inflammation and Metabolism

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### Abstract

**Significance:** Thioredoxin interacting protein (TXNIP) is a member of the arrestin fold superfamily with important cellular functions, including cellular transport, mitochondrial energy generation, and protein cycling. It is the only arrestin-domain protein known to covalently bind to thioredoxin and plays roles in glucose metabolism, inflammation, apoptosis, and cancer.

**Recent Advances:** The crystal structure of the TXNIP-thioredoxin complex provided details about this fascinating interaction. Recent studies showed that TXNIP is induced by endoplasmic reticulum (ER) stress, activates NLR family pyrin domain containing 3 (NLRP3) inflammasomes, and can regulate glucose transport into cells. The tumor suppressor role of TXNIP in various cancer types and the role of TXNIP in fructose absorption are now described.

**Critical Issues:** The influence of TXNIP on redox state is more complex than its interaction with thioredoxin.

**Future Directions:** It is incompletely understood which functions of TXNIP are thioredoxin-dependent. It is also unclear whether TXNIP binding can inhibit glucose transporters without endocytosis. TXNIP-regulated control of ER stress should also be investigated further. *Antioxid. Redox Signal.* 38, 442–460.

**Keywords:** TXNIP, arrestin, thioredoxin, redox state, inflammasome, glucose

### The Arrestin Fold Protein Family

THIOREDOXIN INTERACTING PROTEIN (TXNIP) is a member of a family of proteins believed to have structural similarities. Arrestin fold proteins are predicted to share a common arrestin domain structure and are widely present from single-celled organisms to metazoans, and many proteins in this superfamily play roles in metabolic regulation and intracellular signaling (Habourdin et al, 2013). The arrestin fold superfamily is composed of two main branches: the more ancient alpha-arrestin family and the well-studied beta-arrestin family (Patwari and Lee, 2012) (Fig. 1A).

Sequence-wise, alpha-arrestins, sometimes designated ARRDC for “Arrestin Domain-Containing,” have evolutionarily conserved PY motifs that bind to WW domains

in their C-terminal tails, and beta-arrestins have an alpha-helix that is missing in alpha-arrestins in the arrestin N-terminal domain (Alvarez, 2008). Orthologues of the mammalian ARRDC family have been detected in lower organisms such as yeasts (Arrestin-related trafficking protein ARTs such as Smf1) (Nikko et al, 2008), archaea/bacteria (Spo0m) (Alvarez, 2008), and amoebae (Arrestin-domain containing proteins such as AdcA-F) (Guetta et al, 2010).

In primitive organisms such as *Dictyostelium discoideum*, ARRDC fold proteins respond to stimuli such as cyclic AMP (cAMP) and folate (Mas et al, 2018) resembling mammalian TXNIP, which responds to intracellular carbohydrate and cAMP levels (Patwari and Lee, 2012; Shalev, 2014).

The mammalian arrestin fold superfamily has diverse and essential intracellular functions for cellular homeostasis,

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including intracellular regulation of G-protein coupled receptors (GPCR) (Nabhan et al, 2010; Patwari and Lee, 2012; Shea et al, 2012; Shenoy et al, 2001). “Beta-arrestins” were extensively studied due to their roles in reducing rhodopsin and beta-adrenergic receptor signaling (Benovic et al, 1987; Dorey and Faure, 1977; Pfister et al, 1985). The “alpha-arrestins” also regulate several essential cellular processes in mammals, including glucose transport, fatty acid flux, mitochondrial citric acid cycle, carbohydrate response, extracellular vesicle generation, protein trafficking and ubiquitination, receptor recycling, thermogenesis, and adrenergic signaling (Farooq et al, 2022; Patwari and Lee, 2012; Wedegaertner et al, 2020).

The human alpha-arrestin family is composed of six members: ARRDC 1-5 and TXNIP (Fig. 1A). All alpha-arrestins have predicted structural homology with similar N- and C-terminal arrestin domains (Patwari et al, 2006). The alpha-arrestin protein subfamily members have potentially important tumor-suppressive and tumor growth regulatory roles in several cancers, including breast (Park et al, 2018), skin (Meylan et al, 2021), thyroid (Morrison et al, 2014), and lung cancers (Liang et al, 2021).

Several proteins in the alpha-arrestin family are known to control glucose metabolism in mammals. ARRDC3, which has been linked to human obesity in males, controls insulin receptor signaling in the liver to regulate glucose metabolism (Batista et al, 2020; Patwari et al, 2011). ARRDC4, a close homolog of TXNIP, can control glucose metabolism *in vitro* (Patwari et al, 2009). However, TXNIP is unique as it is the only arrestin fold protein to bind covalently to thioredoxin. Isolated from Vitamin D3-stimulated HL-60 cells, TXNIP was initially named “Vitamin-D-Upregulated-Protein 1” (VDUP1) (Chen and DeLuca, 1994). Following this, the protein was named “thioredoxin binding protein-2 (TBP-2)” from a yeast two-hybrid screen searching for thioredoxin binding proteins (Nishiyama et al, 1999).

The Yodoi lab showed that TBP-2 deficiency enhanced tumor growth factor (TGF)- $\beta$  signaling (Masaki et al, 2012). TBP-2 was also found to improve insulin sensitivity and secretion without affecting obesity (Yoshihara et al, 2010). Later, TXNIP received its widely used name from Bodnar et al in 2002 when the absence of *txnip* was found through positional cloning to cause combined hyperlipidemia in mice (Bodnar et al, 2002).

This review will focus on TXNIP, its unique thioredoxin-binding property and biological functions that may depend on thioredoxin interaction; its regulation of carbohydrate and lipid metabolism; as well as the roles of TXNIP that are not yet well defined mechanistically. We will discuss the possibility that thioredoxin-binding serves as a cellular redox state sensor as a consequence of metabolic status rather than as a thioredoxin inhibitor, thus connecting the thioredoxin-interaction property with the regulation of metabolism by TXNIP.

## Mechanism of the TXNIP-Thioredoxin Interaction

Thioredoxin is a thiol-oxidoreductase that protects cells from oxidative stress (Baba and Bhatnagar, 2018). It is conserved through all species, including bacteria, except for the bacterium *Tropheryma whipplei* because of its parasitic characteristics as obligate intracellular bacteria (Marth, 2015). Thioredoxin reduces protein disulfides through C32 and C35 at its active site (Eklund et al, 1991; Jeng et al, 1994; Patwari and Lee, 2012). The reduced thioredoxin reduces the disulfide bonds of target proteins and becomes oxidized. Nicotinamide adenine dinucleotide phosphate (NADPH)-dependent thioredoxin reductase restores the reduced thioredoxin to start a new cycle (Yamawaki et al, 2003).

Peroxioredoxin is oxidized by hydrogen peroxide and oxidizes a redox-sensitive target protein that is reduced by thioredoxin (Stancill and Corbett, 2021). Methionine sulf-oxide reductases also require reduction by thioredoxin (Dobrowolska et al, 2012). The binding of transcription factor nuclear factor kappa B (NFkB) to DNA requires reduction by thioredoxin (Qin et al, 1995). Humans carry two *thioredoxin (Txn)* genes, *Txn1* in the cytoplasm and *Txn2* in mitochondria. Thioredoxin is critical for mammalian life, and mice with genetic loss of *Txn1* and *Txn2* die shortly after implantation (Matsui et al, 1996; Nonn et al, 2003).

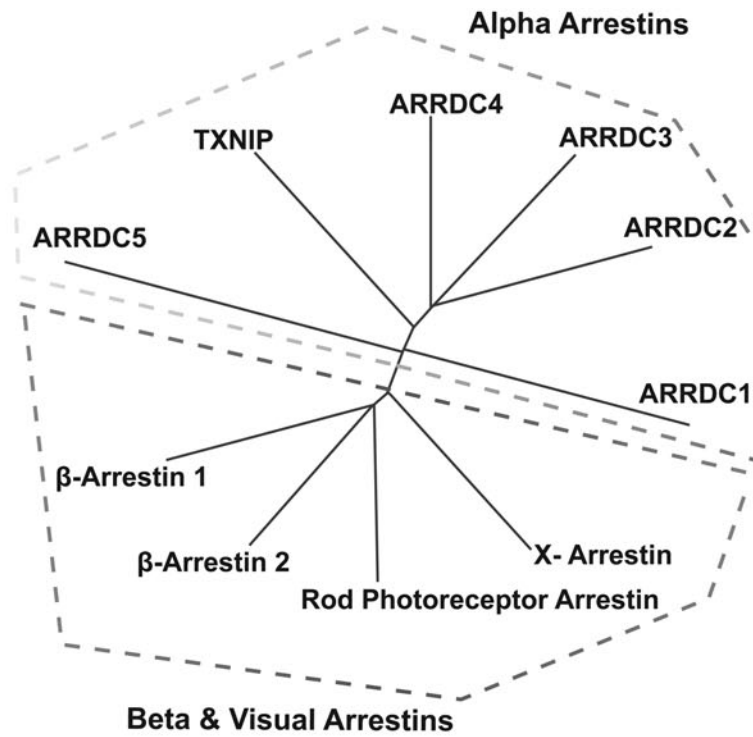
Thioredoxin interaction with TXNIP requires reduced thioredoxin, and TXNIP will not bind to oxidized thioredoxin or catalytic mutants of thioredoxin. On TXNIP, the key cysteine C247 was shown to be an essential residue for thioredoxin interaction by mutational studies in cultured cells (Patwari et al, 2006). This residue appears in TXNIP in later metazoans and is conserved in mammals, and thus the probable thioredoxin-binding function of TXNIP may have evolved for specific purposes (Fig. 1B). In 2014, Hwang et al solved the crystal structure of TXNIP-thioredoxin complex by using a thioredoxin C35A mutant that could not dissociate from its target protein and TXNIP mutant (C120S/C170S/C205S/C267S) that behaved better in *Escherichia coli* expression (Fig. 2).

Because this model was built from *in vitro* studies of purified recombinant proteins where reducing agents were avoided, the second half of the thioredoxin reaction cycle could not be completed and TXNIP was left with a disulfide. Therefore, the structure itself did not support TXNIP as an inhibitor of thioredoxin function. However, it did point out how thioredoxin can reduce TXNIP if its C247 forms an internal disulfide with another cysteine residue. This interaction of TXNIP and thioredoxin may be important for TXNIP stability; thioredoxin may bind to TXNIP and render it more stable, avoiding insulin-induced degradation of TXNIP (Chutkow and Lee, 2011).

The interaction of TXNIP with cytoplasmic *Txn1* has attracted attention, because TXNIP is mostly localized to the cytoplasm and the nucleus. In mitochondria, TXNIP forms a

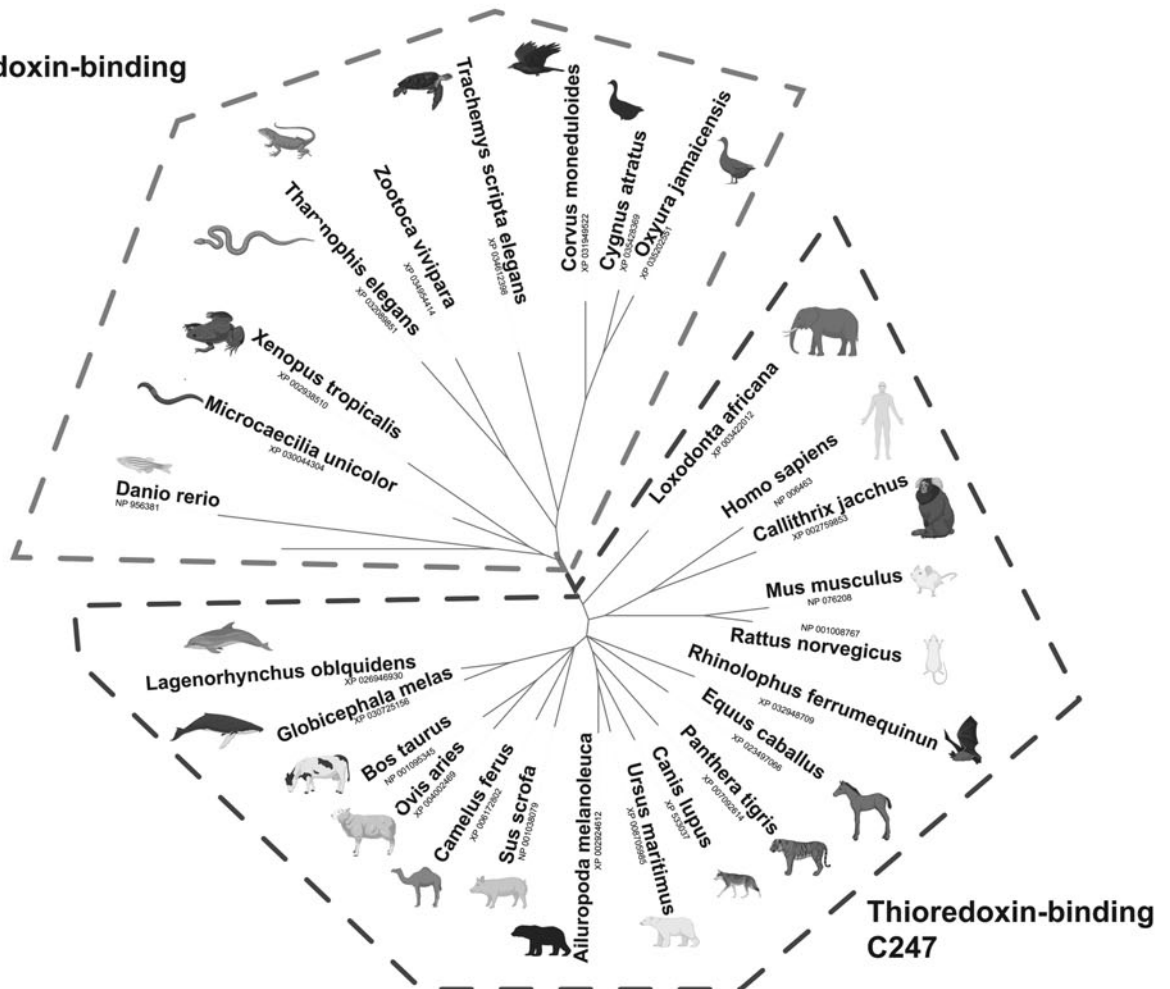
**FIG. 1. TXNIP as a member of the arrestin fold superfamily.** (A) TXNIP is a member of the diverse arrestin fold superfamily that is composed of alpha (on *top*) and beta-arrestin (on *bottom*) branches. (B) The key cysteine in TXNIP (C247) for binding thioredoxin evolved in animals. Mammals bear a C247 cysteine residue, whereas nonmammals such as fish and frogs do not (*light gray*: nonmammals that do not have TXNIP C247 residue, *dark gray*: mammals that have TXNIP C247 residue). TXNIP, thioredoxin interacting protein. (Drawn using iTOL, Letunic et al, 2019 *Nuc. Acid. Res.*, PhytoL and NCBI COBAL, Created using Biorender.com)

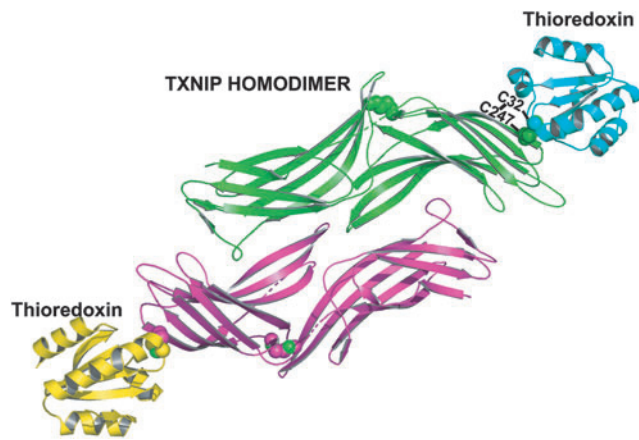
A



B

No Thioredoxin-binding  
C247





**FIG. 2. The TXNIP-thioredoxin interaction.** Crystal structure of the TXNIP dimer-thioredoxin complex, resolution 2.7 Å, balls refer to bonding cysteine residues, *green* and *pink* are beta sheets and disoriented regions of TXNIP, and *yellow* and *cyan* are alpha helices and beta sheets of thioredoxin. Created using PyMOL Molecular Graphics System.

bond with thioredoxin2. This decreases thioredoxin2 binding to apoptosis signal-regulating kinase 1, inducing mitochondrial apoptosis *via* the release of cytochrome c and caspase-3 cleavage (Saxena et al, 2010).

### TXNIP and Inflammasome Activation

A well-characterized biological process that depends on the TXNIP-thioredoxin1 interaction is the activation of inflammasomes. Inflammasomes are cytoplasmic multi-protein structures that are critical in the pathophysiology of multiple diseases and are formed on detection of warning signals for the cell (Broz and Dixit, 2016). The NLR family pyrin domain containing 3 (NLRP3) inflammasome is the most studied inflammasome complex and is composed of NLRP3, apoptosis-associated speck-like protein containing CARD (ASC), and caspase-1 (Zhao and Zhao, 2020). The NLRP3 inflammasome is reported to be associated with multiple infectious diseases, including acquired immune deficiency syndrome (AIDS) by human immunodeficiency virus (HIV)-1 (Pontillo et al, 2010) and tuberculosis (Souza de Lima et al, 2016), as well as metabolic conditions including type 2 diabetes (Lee et al, 2013) and obesity (Vandanmagsar et al, 2011).

Reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress induce NLRP3 inflammasome complex activation, triggering an inflammatory response (Oslowski et al, 2012; Zhao et al, 2020; Zhou et al, 2010). In 2009, Zhou et al (2010) reported that TXNIP may have a pivotal role in ROS-mediated inflammasome activation using cultured cells. They built a model where in normoxic conditions, the TXNIP-thioredoxin interaction renders TXNIP unavailable for NLRP3 interaction (Zhou et al, 2010). On the activation of oxidative stress, TXNIP releases from thioredoxin and attaches to NLRP3 to activate it, and then caspase-1 cleaves interleukin (IL)-1 $\beta$  (Zhou et al, 2010).

This model requires most cellular TXNIP to exist as a covalent complex with thioredoxin in the cytoplasm without stimulation to keep NLRP3 inactive. However, this prereq-

uisite is inconsistent with the reducing environment in the cytoplasm. Therefore, the detailed mechanism of TXNIP involvement in NLRP3 activation needs further investigation. On the other hand, thioredoxin itself may influence NLRP3 activation. *In vitro*, it was shown that thioredoxin1 suppressed the NLRP3 expression and subsequent IL-1 $\beta$  and caspase-1 secretion (Wang et al, 2020). The same article also demonstrated *in vivo* that thioredoxin1 reduced atherosclerosis development by inhibiting NLRP3 inflammasomes (Wang et al, 2020). On the other hand, thioredoxin was shown to enhance NF $\kappa$ B target DNA binding and NLRP3 inflammasome activation independent of TXNIP by another group (Muri et al, 2020).

Multiple inflammatory conditions are linked to the TXNIP-inflammasome axis. High glucose levels promote TXNIP expression and NLRP3 activation in a time- and dose-dependent manner (Gu et al, 2019). In diabetes and atherosclerosis, the NLRP3 inflammasome mediates inflammation through caspase-1 activation and the secretion of pro-inflammatory cytokines IL-1 $\beta$ /IL-18 (Grebe et al, 2018; Schroder et al, 2010; Sun et al, 2017; Zhou et al, 2010). The ROS-TXNIP-NLRP3 biological axis may also participate in tubular injury of diabetic neuropathy (Han et al, 2018). Hepatic inflammation due to poor diet was linked to the TXNIP-NLRP3 axis (Mohamed et al, 2018).

TXNIP ablation improved high-fat diet-induced hepatosteatosis and inflammation by regulating the TLR2-NLRP3 inflammasome pathway (Mohamed et al, 2018). TXNIP was also found to play a role in fructose-induced inflammasome activation in the liver (Zhang et al, 2015); rats given fructose-containing drinking water for 8 weeks had elevated TXNIP and NLRP3 inflammasome levels in the liver. Interestingly, in *txnip*-silenced hepatocytes, fructose did not induce inflammasome activation (Zhang et al, 2015). The TXNIP-NLRP3 pathway also has effects on the colon.

For instance, the NLRP3 inflammasome may play a role in ulcerative colitis development (Zhen and Zhang, 2019), and TXNIP was found to be important for inflammation of colonic mucosa (Takahashi et al, 2007). In concordance with that concept, a flavonoid was shown to inhibit macrophage NLRP3 inflammasome activation recently in a model of ulcerative colitis by suppressing the TXNIP pathway (Liu et al, 2020).

The TXNIP-NLRP3 inflammasome plays roles in diverse organ systems, including the cardiovascular, respiratory, and nervous systems. For example, TXNIP activates the NLRP3 complex in myocardial ischemia/reperfusion injury in an ROS-dependent fashion (Liu et al, 2014). An essential part of the inflammatory response, macrophage activation, is also regulated by the TXNIP-NLRP3/inflammasome intracellular pathway (Lutz et al, 2016). The TXNIP-thioredoxin complex together with the nuclear factor erythroid 2-related factor 2 (NRF2) can also affect redox balance in a mouse model of Alzheimer's disease, and antioxidant treatment can suppress TXNIP-NLRP3 inflammasome activation by NRF2 induction (Wang et al, 2019).

The involvement of TXNIP in inflammasome complexes is not limited to the NLRP3 complex. A decrease in expression of the NLRP1 complex proteins was observed recently in human  $\beta$  cells treated with the small-molecule TXNIP inhibitor SRI-37330, which is a potential drug candidate for diabetes (Thielen et al, 2020).

### TXNIP and Regulation of Glucose Metabolism by a Cellular Glucose Autoregulatory Circuit

Beyond its thioredoxin interacting ability, TXNIP regulation of metabolism in mouse models is clearly established. The first loss-of-function model arose when Bodnar et al (2002) identified a truncated *txnip* gene using positional cloning analysis of a combined hyperlipidemia mouse. Later, four other groups independently generated TXNIP knockout (KO) animals (Hui et al, 2008; Lee et al, 2005; Oka et al, 2006; Yoshioka et al, 2007), including two conditional deletion lines. Consistently, TXNIP global KO animals were reported to exhibit increased fasting plasma triglycerides, free fatty acids, ketone bodies, and hypoglycemia. These phenotypes were strong enough to be observed in fed mice in some cases (Oka et al, 2006; Sheth et al, 2005).

In 2007, Parikh et al made the discovery that TXNIP negatively regulates glucose uptake in peripheral tissues. Overexpressing TXNIP in cultured adipocytes reduced glucose uptake, whereas knocking down *txnip* enhanced glucose uptake. The lack of TXNIP also promoted adipogenesis in cultured cells (Chutkow and Lee, 2011; Chutkow et al, 2010), which was consistent with a higher adiposity in mice without *txnip* (Chen et al, 2008a; Chutkow et al, 2010; Sheth et al, 2005). TXNIP regulation of glucose uptake may explain the major metabolic phenotypes observed in mice.

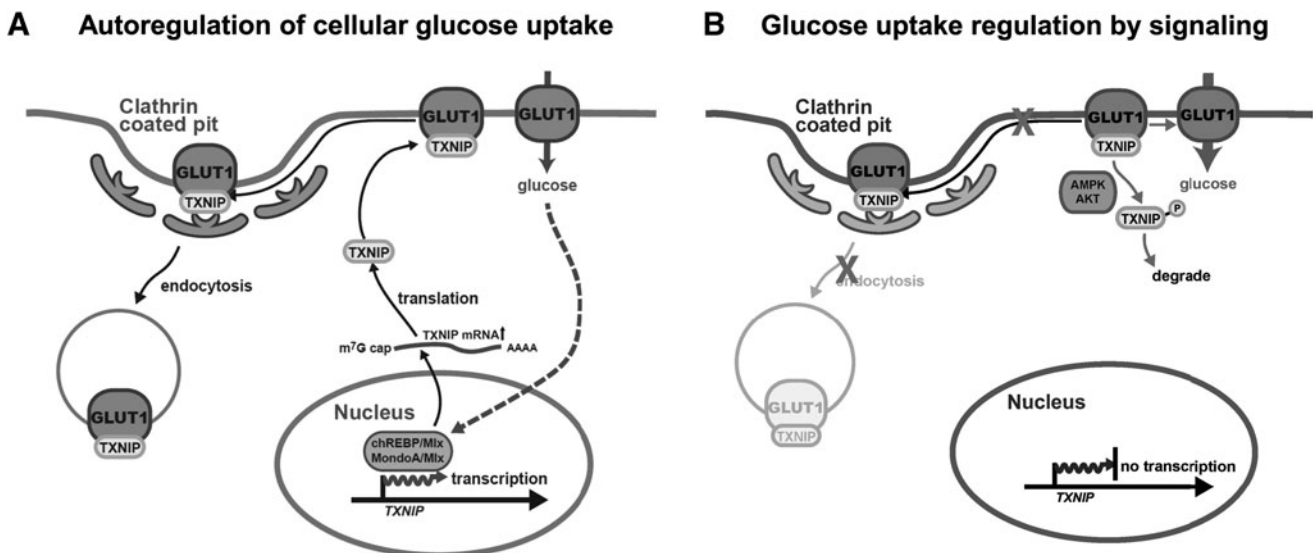
The absence of TXNIP leads to more glucose taken up into peripheral tissues, and thus the TXNIP KO animal becomes hypoglycemic whereas hyperlipidemia follows due to excess intracellular glucose. Since excess glucose can be stored as fat (Chutkow et al, 2010) or secreted as lactate after glycolysis, this could result in a higher blood lactate level (Bodnar et al, 2002; Oka et al, 2006; Sheth et al, 2005). By allowing peripheral tissues to take up more glucose, *txnip* deletion can protect animals against high-fat diet-induced and genetically induced insulin resistance (Chen et al, 2008a;

Chutkow et al, 2010; Hui et al, 2008). In 2009, Patwari et al demonstrated that TXNIP regulation of glucose uptake is independent of its interaction with thioredoxin using the TXNIP C247S mutant. As described earlier, a closely related alpha arrestin family member, ARRDC4, which does not possess C247 and does not interact with thioredoxin, also has the ability to inhibit glucose uptake (Patwari et al, 2009).

Further, *TXNIP* is one of the top genes regulated by glucose availability on the transcriptional level *via* carbohydrate response element binding protein (ChREBP)/Mlx and MondoA/Mlx (Cha-Molstad et al, 2009; Pongvarin et al, 2015; Stoltzman et al, 2008). These transcription factors bind to the carbohydrate response element on the *TXNIP* promoter to induce gene expression. This completes a cellular autoregulatory circuit for adjusting how much glucose should be allowed into the cell at a time. TXNIP protein is rapidly produced on glucose stimulation to reduce further glucose influx (Fig. 3A).

Recently, TXNIP was shown to facilitate fructose absorption in the small intestine (Dotimas et al, 2016; Shah et al, 2020). A high-fructose diet induces both *txnip*, *solute carrier family 2 member 2 (slc2a2)*, and *slc2a5* gene expression in the intestine. Depending on how fructose was supplied (either in solid diet or in drinking water) and the type of TXNIP KO used (total KO or intestinal KO), upregulation of *slc2a5* can be dependent on TXNIP. In intestinal-specific *txnip* deletion, TXNIP was found to be required for the apical localization of GLUT5.

In contrast to the glucose-ChREBP/MondoA/Mlx-TXNIP negative feedback loop, the absence of TXNIP reduces fructose uptake instead of promoting it, and the dependence of TXNIP induction by fructose on ChREBP/Mlx has yet to be clarified (Kim et al, 2017). The studies also showed that energy-rich diets promote both *txnip* and *slc2a5* expression and fructose absorption. Given the important role that



**FIG. 3. A TXNIP autoregulatory pathway of cellular glucose.** Autoregulatory pathway of cellular glucose. (A) Glucose metabolites induce *Txnip* transcription *via* ChREBP/Mlx or MondoA/Mlx. TXNIP protein binds to GLUT1 on the plasma membrane and facilitates GLUT1 endocytosis *via* the clathrin pathway. (B) Activated AMPK from energy stress or activated Akt from growth factor stimulation phosphorylates TXNIP on Ser308, leading to its dissociation from GLUT1. This results in the immediate inhibition of GLUT1 endocytosis and acute increase in glucose uptake. AMPK, AMP-activated protein kinase; ChREBP, carbohydrate response element binding protein; GLUT1, glucose transporter 1.

fructose may play in metabolic disease, this finding indicates a potentially important interaction between glucose and fructose metabolism in the intestine epithelium.

### Mechanism of TXNIP Regulation on Glucose Transport

In 2013, Wu et al discovered that TXNIP is an adaptor protein for clathrin-dependent endocytosis of the glucose transporter GLUT1. TXNIP contains a dileucine motif (L351, L352) in its flexible C-terminal tail that serves as either a clathrin or an AP2 binding site. A mutation of these two residues abolishes the clathrin-coated pit interaction, but not plasma membrane localization of TXNIP. However, the removal of the clathrin-interaction motif from TXNIP was sufficient to decrease the endocytosis rate of GLUT1. It was later observed that TXNIP also serves as an adaptor for GLUT2, GLUT3, and GLUT4 (Waldhart et al, 2017); GLUT4 is likely the physiological target of TXNIP within adipose tissue and muscle.

GLUT1-4 is a facilitator, thus glucose flows through these transporters in the direction of a high-to-low concentration with no other energy requirement. Cytoplasmic-free glucose concentration is usually kept lower than that of the blood by the rapid formation of glucose-6-phosphate. Therefore, how much glucose passes through these transporters depends on how many are present on the cell surface. Hence, higher expression of TXNIP will result in more transporter endocytosis and thus fewer available transporters on the plasma membrane for glucose. In the absence of TXNIP, endocytosis of transporters may be slowed, thus permitting greater glucose influx. It remains unclear whether the binding of TXNIP can inhibit glucose transporters even without endocytosis.

The interaction of TXNIP with both plasma membrane and glucose transporters requires phosphoinositides (Dykstra et al, 2021; Waldhart et al, 2017). TXNIP interaction with liposomes containing negatively charged phosphoinositides can be detected using a liposome-based floating assay. Using reconstituted GLUT1 in PI(4,5)P<sub>2</sub>-containing lipid nanodiscs and recombinant TXNIP, this interaction can be quantified with isothermal titration calorimetry. It was found that the bulk of binding energy comes from the ionic interaction between the acidic lipid and the basic residues K233 and R238 on TXNIP. However, there may still be some specificity coming from GLUT1-TXNIP interaction since there was no detectable interaction between rat GLUT5 and TXNIP using the same system. Together with the *in vivo* fructose uptake data, this suggests that the physical interaction between TXNIP and GLUT5 may be indirect (Dykstra et al, 2021; Waldhart et al, 2017).

These findings illustrate a major difference between beta-arrestins and TXNIP. First, beta-arrestins reside in the cytoplasm under basal conditions whereas TXNIP is already found on the plasma membrane. Second, beta-arrestin-GPCR interactions are initiated by beta-arrestins binding to the phosphorylated GPCR tails whereas the interaction with phospholipids comes later to stabilize the complex. On the other hand, this lipid interaction is crucial for TXNIP localization to the membrane to initiate interaction with GLUT1. Sequence-wise, R238 but not K233 is conserved in ARRDC3 and ARRDC4. It remains to be seen whether the basic pocket for lipids is conserved on the three-dimensional structural level for all alpha-arrestins.

In addition to the plasma membrane location, TXNIP is found in fast trafficking vesicles close to the plasma membrane, presumably in various stages of endosomes although their exact nature has not yet been confirmed. Mechanistically, this raises the possibility that TXNIP can bind to inositol lipids other than PI(4,5)P<sub>2</sub>. If so, whether a differential affinity toward the different types of head groups determines the binding status of TXNIP onto different vesicles and the role that TXNIP plays in glucose transporter recycling are areas in need of further study.

### Signaling Intervention

Since cells constantly respond to intracellular metabolic needs and extracellular signals, both should be able to modify the TXNIP-enabled autoregulatory circuitry on glucose uptake (Fig. 3B). Given this, not surprisingly, it turns out that TXNIP also serves as a signaling hub. Both AMP-activated protein kinase (AMPK) and Akt phosphorylate TXNIP on S308, causing TXNIP to dissociate from glucose transporters, leading to an immediate increase in glucose uptake (Waldhart et al, 2017; Wu et al, 2013). Purified recombinant TXNIP from *E. coli* has its C-terminal tail extended away from the N-terminal arrestin-fold domains (Dykstra et al, 2021).

How phosphorylation triggers the release of TXNIP from glucose transporters remains currently unknown. Many phosphorylation sites have been detected on the C-terminal tail of TXNIP (phosphosite), and perhaps some of these sites have the same effect as pSer308 in terms of affecting glucose uptake.

On a physiological level, TXNIP regulation on GLUT4 downstream of insulin-phosphoinositide 3-kinases (PI3K)-Akt pathway exemplifies insulin control of GLUT4 endocytosis, a pathway that was previously thought to be passive since GLUT4 carries its own endocytosis signal (F<sup>5</sup>QQI and LL<sup>490</sup>) (Blot and McGraw, 2006). However, during insulin stimulation, transporter endocytosis must be stopped to maximize glucose flux. This is achieved through TXNIP phosphorylation, implying a major contribution of TXNIP as a signal-dependent endocytosis facilitator.

In diabetic patients, if the signaling strength through PI3K-Akt is weakened, insufficient TXNIP might be phosphorylated and the increase in glucose uptake would be dampened, resulting in the high blood glucose phenotype observed in insulin resistance. It is also possible that high blood glucose causes excess TXNIP expression to resist action of insulin-Akt on the single-cell level, contributing further to the insulin resistance phenotype.

*TXNIP* transcription is regulated by Akt. In the absence of insulin signaling (fasting), Akt is autoinhibited (Chu et al, 2020) and its substrate, forkhead box O (FOXO), stays within the nucleus to drive *TXNIP* expression (Noblet et al, 2021), thus inhibiting glucose uptake in insulin-responsive tissues. TXNIP has been reported to increase after fasting in muscles (Waldhart et al, 2017) and the liver (Oka et al, 2006). On insulin stimulation, activated AKT phosphorylates FOXO, which is then retained in the cytoplasm by 14-3-3 binding, and TXNIP expression is decreased, allowing more glucose influx (Hong et al, 2016; Tzivion et al, 2011). A similar action of AMPK on *TXNIP* gene and protein expression has also been reported (Shaked et al, 2011).

### Glucose-Dependent Cellular Effects of TXNIP in Mitochondria

Genetic deletion of *TXNIP* results in both functional and structural mitochondrial defects in cardiac and skeletal muscle. Mechanistically, there may be multiple ways in which TXNIP affects mitochondria (Yoshioka et al, 2012; Yoshioka et al, 2007). One way glucose can affect mitochondria function is *via* rigidifying membranes by affecting their lipid composition (Waldhart et al, 2021). This change in lipid ratios can be a direct consequence of excess tissue glucose uptake in the absence of TXNIP, because it can be rescued by using a ketogenic diet (Elshaer et al, 2017; Waldhart et al, 2021).

*De novo* lipogenesis from glucose primarily generates C16:0, C18:0, C18:1, and other shorter and more saturated acyl-chains, which effectively reduces the percentage of dietary polyunsaturated long chain fatty acids (PUFAs) that are critical for optimal mitochondrial function. Functions of mitochondrial electron transport chain complexes, including the formation of the super complex as well as expression of other proteins (such as uncoupling protein 1 [UCP1]) (Hoang et al, 2013), require correct membrane lipid composition and fluidity.

This is illustrated by Barth syndrome in mutations of tafazzin, an enzyme involved in cardiolipin maturation (Gonzalez, 2005; Lu et al, 2016; Xu et al, 2006). In the case of brown adipose tissue (BAT), the functional defect becomes measurable under cold shock stress that requires a fast and dramatic increase in mitochondrial metabolic flux. Membrane lipid change is probably not the only cause of mitochondrial defects observed in TXNIP KO mice. In the BAT study, there are many gene expression changes in TXNIP KO not rescued by a ketogenic diet (Waldhart et al, 2021). This suggests there are non-glucose-dependent functions of TXNIP that require further investigation. Efforts also have been made in developing small molecules to inhibit TXNIP expression to manage insulin resistance (Li et al, 2017; Thielen et al, 2020).

### Cell Cycle

Primary mouse embryonic fibroblasts (MEFs) isolated from TXNIP KO animals demonstrate Warburg-like metabolism: higher glucose uptake, more lactate production, less glucose oxidation, and faster cell doubling (Hui et al, 2008). Faster cell growth is made possible by having more glucose available to generate building blocks. In addition, in order for a cell to be divided into two daughter cells, mitochondria must be broken down and redistributed (Carlton et al, 2020). Faster cell division necessitates less tubular forms of mitochondria and thus less efficient oxidative phosphorylation (OXPHOS) and more reliance on glycolysis to provide ATP.

Using synchronized cells to profile proteins and metabolites during different stages of cell division, it was found that TXNIP expression was inhibited whereas GLUT1 expression increased at the beginning stages of the cell cycle to increase glucose uptake (Lee et al, 2017). Therefore, in the absence of oncogenic mutations, the availability of metabolites alone can potentiate cell growth in culture. *In vivo*, this may be relevant to tumor suppression by TXNIP. TXNIP KO on the C3 strain of mice developed hepatocellular carcinoma (Sheth et al, 2006), and TXNIP KO mice from another group

were more susceptible to diethylnitrosamine-induced hepatocarcinogenesis (Kwon et al, 2011).

TXNIP absence also accelerates liver regeneration after a partial hepatectomy (Kwon et al, 2011). Together with the fact that mitochondrial oxidative capacity is decreased in the absence of TXNIP, glucose availability may directly influence cell replication. Therefore, it is not surprising that for rapidly dividing cells in some cancers, overexpressing TXNIP can be “tumor suppressive” (Chen et al, 2020). The earlier interpretation from the metabolite availability perspective is consistent with many reports that low TXNIP expression is associated with poor prognosis (Masutani, 2021) (also see later discussion). In addition, TXNIP protein itself can participate in cell cycle regulation (Jeon et al, 2005; Kamitori et al, 2018).

### ER Stress

The ER stress partners with oxidative stress in the pathophysiology of several diseases (Chong et al, 2017). Overloading of ER stress can cause calcium release and oxidative stress, leading to inflammation (Zhang and Kaufman, 2008). The ER stress plays an essential role in type 1 and type 2 diabetes (Eizirik et al, 2008) due to its association with inflammation (Zhang and Kaufman, 2008). TXNIP was found to be the link that connects inflammation to ER stress (Osowski et al, 2012). Peripheral blood mononuclear cells that were obtained from type 2 diabetes patients also showed increased TXNIP expression (Szipigel et al, 2018).

This finding also holds true *in vitro*; induction of ER stress in human monocyte-derived macrophages triggers TXNIP, NLRP3, and IL-1 $\beta$  production (Szipigel et al, 2018). TXNIP expression also regulates glucose metabolism in breast cancer cells; estrogen receptor activates the unfolded protein response (UPR) and represses TXNIP to divert glucose metabolism (Wang and Chen, 2021).

UPR is a signaling pathway that helps ER to retain its functionality. UPR is induced by stress factors such as inositol-requiring enzyme 1 (IRE1), the PERK-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) (Hetz, 2012). The ER stress caused by chemicals such as thapsigargin and tunicamycin as part of UPR induces TXNIP expression in cultured INS-1 832/13 cells as well as primary human and mouse islets (Lerner et al, 2012; Osowski et al, 2012). This induction is mostly *via* PERK and IRE1 pathways. TXNIP is required for the expression of IL-1 $\beta$  downstream of ER stress, leading to ER stress-mediated cell death.

Together with *in vivo* studies, it appears that the absence of TXNIP can protect  $\beta$  cells from apoptosis, increase  $\beta$  cell mass, and enhance insulin secretion (Chen et al, 2010; Chen et al, 2008a; Chen et al, 2008b; Hui et al, 2004; Oka et al, 2009; Yoshihara et al, 2010). The ER stress can reduce glucose uptake and lactate production (Wang et al, 2011); in this case, the authors used IL-3-dependent Bak<sup>-/-</sup>Bax<sup>-/-</sup> cells to avoid ER stress-induced cell death and explained the change in glucose uptake with reduced surface level of GLUT1, consistent with the increase in TXNIP previously reported. Therefore, ER stress-induced TXNIP expression results in decreased glucose uptake. However, the lack of glucose can lead to induction of glucose-regulated proteins that are involved in UPR (Ellgaard et al, 2018; Lee, 2014; Lee, 1992).



Folding proteins properly in the ER involves shuffling disulfide bridges. The sources of reducing power in ER to support protein folding are still unclear but most likely require glucose (further discussion below). Therefore, the glucose-TXNIP axis plays a role in the ER stress response, and further studies are needed to delineate the causal relationship under both mild and extreme ER stress conditions, particularly *in vivo* in metabolism and not in a chemical-induced manner.

#### TXNIP as Thioredoxin Inhibitor or Effector

Here, we shall discuss whether TXNIP acts as an inhibitor or effector of thioredoxin, and how its regulation of glucose metabolism can also affect cellular redox state. Glucose availability indirectly affects cellular redox state for the following reasons. The cytoplasm is generally thought to have a reduced environment with 1–10 mM glutathione (GSH) as the most abundant reducing agent. The cellular concentration of GSH is much higher than thioredoxin, but both have the same reducing potential (Schafer and Buettner, 2003). The regeneration of both GSH and thioredoxin uses the reducing potential in NADPH that can be generated *via* different pathways (Fig. 4).

There are separate pools of NADPH in the cytoplasm, ER, and mitochondria (Lewis et al, 2014; Maddocks et al, 2014; Mari et al, 2009; Rogoff et al, 2010). However, regardless of their location, the ultimate reducing power in NADPH comes from energy stored in the carbon–carbon bonds such as glucose, created by photosynthesis. As shown in Figure 4, aldehyde dehydrogenase 1 family member (ALDH1)L1/2, methylenetetrahydrofolate dehydrogenase cyclohydrolase and formyltetrahydrofolate synthetase 1 (MTHFD1/1L), function in 1-carbon metabolism; glucose-6-phosphate dehydrogenase (G6PD), phosphogluconate dehydrogenase (PGD), and hexose-6-phosphate dehydrogenase/glucose 1-dehydrogenase (H6PD) are directly downstream of glucose usage; isocitrate dehydrogenase [NADP(+)] (IDH1/2) and

malic enzyme (ME)1/3 work in central carbon shuttling; and nicotinamide nucleotide transhydrogenase (NNT) uses the proton gradient to generate NADPH. Therefore, any process that affects cellular glucose availability can affect NADPH levels, thus affecting the available free thiol pool and ROS neutralization.

Given this reducing environment, TXNIP is most likely a thioredoxin effector, not inhibitor. That is, stress-induced oxidation of C247 in TXNIP is rescued by thioredoxin. To be a thioredoxin inhibitor, TXNIP needs to satisfy the following conditions in the cell: (1) maintain internal disulfide bonds so it can bind thioredoxin; (2) the cell contains at least equal amounts of oxidized TXNIP protein and reduced thioredoxin protein; (3) once the disulfide is transferred to thioredoxin, thioredoxin is not efficiently reduced by thioredoxin reductase; or (4) the reduced TXNIP has to become oxidized quickly to match the speed that thioredoxin is reduced by thioredoxin reductase to keep thioredoxin inhibited.

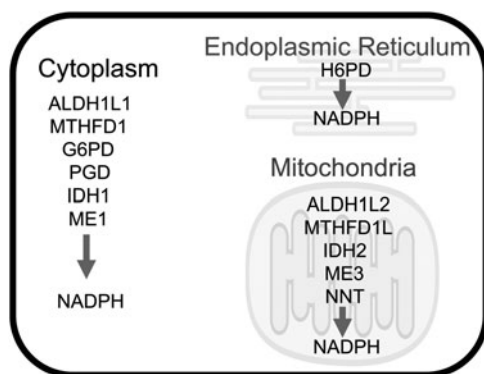
However, TXNIP is, undoubtedly, an important substrate of thioredoxin. Therefore, thioredoxin availability will affect TXNIP function, including its regulation of glucose transport and thus cellular NADPH concentration. Depending on the biological system, if TXNIP is the major effector of thioredoxin in the system, then it is possible that by having to rescue TXNIP, less free reduced thioredoxin is available for its other targets such as detoxifying H<sub>2</sub>O<sub>2</sub> by peroxiredoxins (Buettner et al, 2013) or less NADPH is available for glutathione reductase to maintain the GSH pool (Fig. 5).

We stress the point that the redox outcome will depend on the biological context. This is illustrated by the following evidence: The absence of TXNIP does not appear to affect liver thioredoxin level or activity (Sheth et al, 2005), even though there is a higher GSH:glutathione disulfide (GSSG) ratio (Hui et al, 2004). Thioredoxin activity is not different between TXNIP KO and wildtype mice in basal conditions (Sheth et al, 2005; Yoshioka et al, 2012) nor in human primary *TXNIP*<sup>-/-</sup> cells compared with controls (Katsu-Jiménez et al, 2019). However, TXNIP KO mice have a significant increase in the ratio of NADH to NAD<sup>+</sup> in the fed state (Sheth et al, 2005). In cardiomyocytes, the TXNIP C247S mutation did not affect basal levels of either TXN1 or TXN2 activity or the GSH:GSSG ratio.

On the other hand, with streptozotocin-induced diabetes and acute myocardial infarction, TXNIP wildtype hearts have a lower GSH:GSSG ratio, lower TXN2 activity, but the same TXN1 activity as C247S hearts (Mukai et al, 2021; Nakayama et al, 2021). This illustrates the interconnection of cellular pools of reducing power. Depending on the biological context, TXNIP absence may be associated with reduced ROS such as in lung fibroblasts or higher ROS such as in transplanted bone marrow cells (Jung et al, 2013; Lee et al, 2005). Thus, the influence of TXNIP on cellular redox state is insufficiently explained by its interaction with thioredoxin. It may be a combination of glucose availability, NADPH production and usage, and ROS production that depends on the health of and flux through mitochondria and other organelles such as peroxisomes as well as the cell type (Fig. 5).

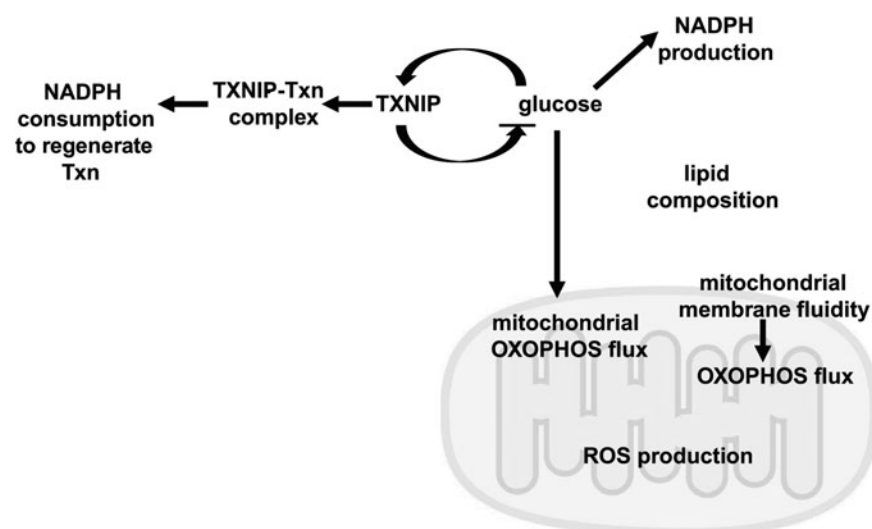
#### Other Functions of TXNIP

Earlier, we discussed TXNIP's interaction with thioredoxin and TXNIP's regulation of glucose in detail. Other



**FIG. 4. Enzymes that generate NADPH at different locations in cell.** ALDH1L1/2, aldehyde dehydrogenase 1 family member L1/2; G6PD, glucose-6-phosphate dehydrogenase; H6PD, hexose-6-phosphate dehydrogenase/glucose 1-dehydrogenase; IDH1/2, isocitrate dehydrogenase [NADP(+)]; ME1/3, malic enzyme 1/3; MTHFD1/1L, methylenetetrahydrofolate dehydrogenase cyclohydrolase and formyltetrahydrofolate synthetase 1; NADPH, nicotinamide adenine dinucleotide phosphate; NNT, nicotinamide nucleotide transhydrogenase; PGD, phosphogluconate dehydrogenase.





**FIG. 5. Potential mechanisms by which TXNIP can affect cellular redox state.** Visual representation of our current knowledge regarding the potential ways in which TXNIP affects cellular redox state. TXNIP and glucose form an auto-feedback loop together, regulating each other. Glucose is needed to produce NADPH such as *via* the pentose phosphate pathway. Too much glucose can lead to increased ROS production by the mitochondria in two ways: (1) Increased metabolic flux through mitochondria increases ROS production; (2) excess glucose rigidifies mitochondrial membranes, leading to less efficient OXOPHOS flux and increased ROS. NADPH is consumed by intrinsic cellular defense mechanisms to counter ROS. NADPH is also consumed when oxidized Txn is regenerated. ROS, reactive oxygen species; Txn, thioredoxin.

functions of TXNIP such as its involvement in autophagy *via* binding to the regulated in development and DNA damage responses 1 (REDD1), a mammalian target of rapamycin (mTOR) regulator, will be discussed in the next section in the context of muscle function. Here, it is worth mentioning that in many cultured cells, TXNIP has a prominent nuclear localization (Nishinaka et al, 2004), though plasma membrane and vesicular TXNIP localization are also visible in cultured cells that grow in tight clusters. The exact function of nuclear TXNIP remains unclear.

A broad proteomics study identified proteins that bind to TXNIP using the proximity-based labeling method to understand the cellular functions of TXNIP and their redox-dependency using C247S mutation (Forred et al, 2016). Many protein interactions with TXNIP were nuclear, and some are redox-dependent since protein binding was disrupted by the C247S mutation. Nuclear TXNIP function may be a consequence of TXNIP stress response or redox sensing. Moreover, many interactions were glucose-dependent and triggered by hyperglycemia (Forred et al, 2016). However, not all interactions of TXNIP are glucose-triggered. The primary effect of TXNIP on the myocardium may arise from its oxidative actions (Domingues et al, 2021).

Given the evolution of TXNIP's ability to bind to thioredoxin, it is not surprising that TXNIP bears both thioredoxin-dependent and -independent regulatory roles (Cao et al, 2020) (Table 1). Studies suggest that induction of oxidative stress, inflammasomes, and cardiac hypertrophy are thioredoxin binding-dependent functions of TXNIP, whereas TXNIP's regulation of glucose uptake and insulin secretion are not thioredoxin binding-dependent (Spindel et al, 2012). Other alpha-arrestins in the family with a high sequence homology to TXNIP do not bind to thioredoxin, but they still have mammalian metabolic roles (Patwari and Lee, 2012; Patwari et al, 2006).

TXNIP itself also has diverse metabolic roles, including regulation of hyperlipidemia, hypoglycemia, gluconeogenesis, glucotoxicity, glucose/fructose uptake, and feeding response (Bodnar et al, 2002; Chutkow et al, 2008; Dotimas et al, 2016; Parikh et al, 2007; Patwari et al, 2009; Shah et al, 2020; Shalev, 2008; Sheth et al, 2005). However, it is not yet understood whether all of these metabolic roles of TXNIP are regulated by thioredoxin binding. Numerous studies have mutated the key cysteine C247 to serine (C247S mutation) in the TXNIP protein *in vitro* to render it unable to bind thioredoxin.

TABLE 1. THIOREDOXIN-DEPENDENT AND -INDEPENDENT FUNCTIONS OF THIOREDOXIN INTERACTING PROTEIN

<i>Thioredoxin-dependent</i>	<i>Thioredoxin-independent</i>
Hepatic gluconeogenesis (Chutkow et al, 2008)	Cellular glucose uptake (Patwari et al, 2009)
Inflammation/NLRP3 inflammasome activation (Zhou et al, 2010)	Angiogenesis (Dunn et al, 2014)
Mitochondrial energy metabolism (Richards et al, 2017; Yoshioka and Lee, 2014)	Hypoxia-inducible factor transcriptional activity (Farrell et al, 2010)
Cardiac function/hypertrophy (Yoshioka et al, 2004), myocardial infarction (Nakayama et al, 2021)	Induction by glucose (Richards et al, 2017)
Ventricular functional reserve (Mukai et al, 2021)	Survival of cone photoreceptors (Xue et al, 2021)

Using this approach, it was shown that both TXNIP and the TXNIP-C247S mutation inhibit glucose uptake in 3T3-L1 adipocytes even though TXNIP-C247S samples had high thioredoxin activity (Patwari et al, 2009; Patwari et al, 2006). On the other hand, wildtype TXNIP protein rescued hepatocyte glucose production with a genetic *TXNIP* deletion, whereas the TXNIP-C247S mutant protein could not (Chutkow et al, 2008). These studies suggest that some metabolic functions of TXNIP may be redox-dependent, whereas others are not.

### TXNIP and Its Actions on Diverse Tissues

*Txnip* expression is modulated by glucose (mediated by carbohydrate-response elements and associated transcription factors Mlx/MondoA), glutamine, fatty acids, and adenosine-containing molecules (Yu et al, 2010). The level of TXNIP is under tight regulation of physiological conditions. In HeLa cells, cycloheximide treatment causes rapid protein turnover (half-life <1 h) of the pre-existing endogenous TXNIP protein (Yu et al, 2010). TXNIP is expressed almost ubiquitously except in the central nervous system, where the expression is low (Tsubaki et al, 2020).

Because TXNIP is expressed in various tissues, including skeletal muscle, adipose, pancreas, and heart, it is not surprising that TXNIP appears to play roles in diverse disease processes (Fig. 6). Interesting insight on the role of TXNIP in

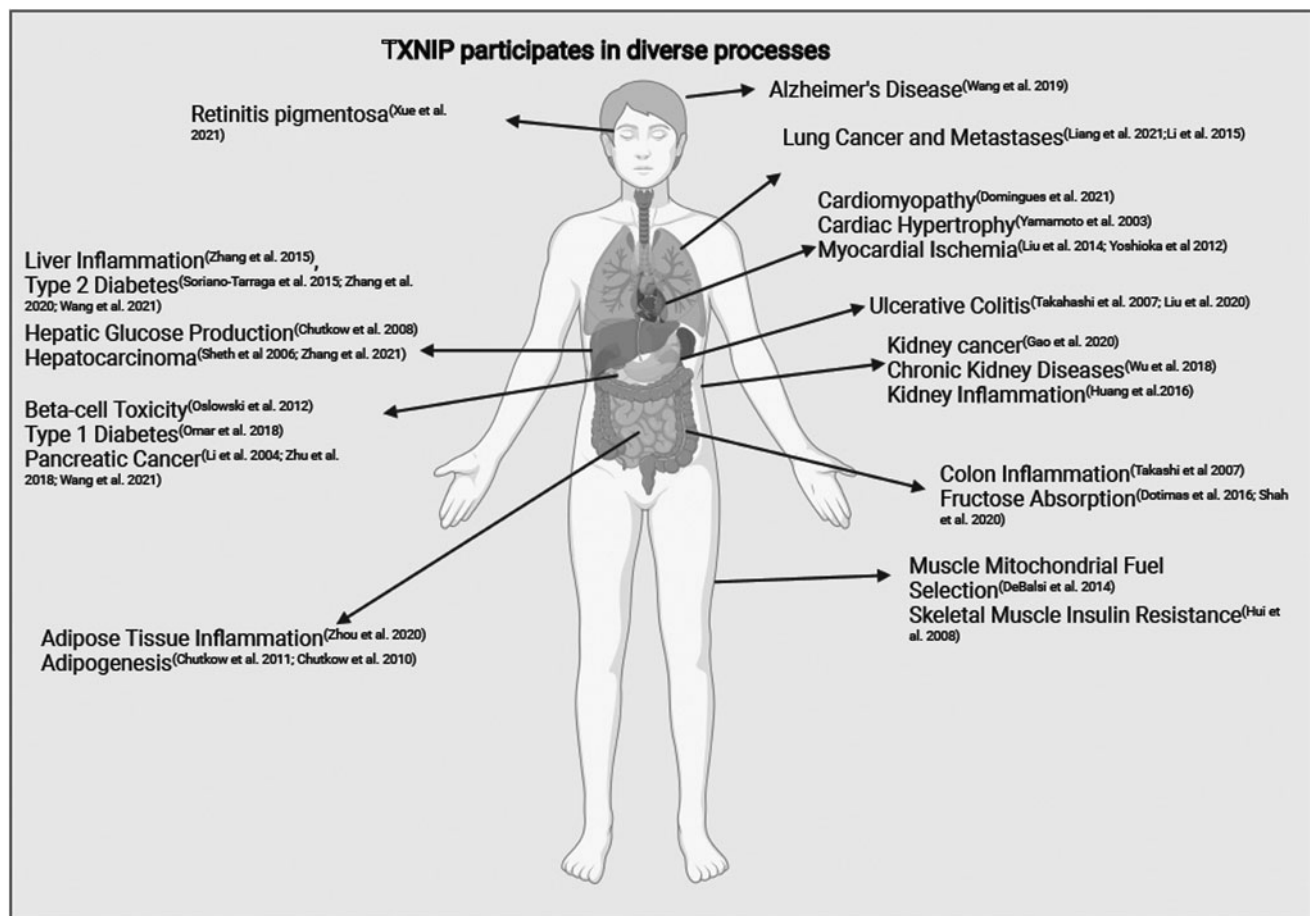
human metabolism comes from new genetic investigations. Three members of a family were homozygous for a *txnip* mutation that ceases TXNIP protein expression; the loss of TXNIP resulted in lactic acidosis and low serum methionine levels (Katsu-Jiménez et al, 2019). *TXNIP*-deficient fibroblasts and myoblasts from these patients could not utilize pyruvate for OXPHOS.

Consistent with this fascinating human finding, animal studies also showed that TXNIP KO mice are deficient in pyruvate utilization by the liver (Chutkow et al, 2008) and that lactate formation was higher in the hearts after ischemia-reperfusion injury (Yoshioka et al, 2012).

Here, we will review published data on TXNIP in specific tissues. These phenotypes are most likely a combination of known cellular functions of TXNIP discussed previously and yet to be discovered functions of TXNIP.

### Heart

TXNIP's oxidative actions may determine its effect on the myocardium (Domingues et al, 2021). It is known that inhibiting endogenous thioredoxin in the heart increases oxidative stress and cardiac hypertrophy (Yamamoto et al, 2003). It was also recently shown that the C247S mutation of TXNIP in heart tissue protects mice from myocardial oxidative stress caused by streptozotocin-induced diabetes (Mukai et al, 2021). This single C247S amino acid mutation



**FIG. 6. TXNIP participates in diverse processes.** TXNIP has reported roles in many pathophysiological processes. (Created in BioRender.com)

in the myocardium allowed the preservation of the anti-oxidative effects of thioredoxin in cardiomyocytes and reduced the adverse effects of myocardial infarctions (Nakayama et al, 2021).

TXNIP plays a fundamental role in myocardial energy homeostasis (Domingues et al, 2021; Yoshioka et al, 2012; Yoshioka et al, 2007). Single-nucleotide polymorphisms in the *txnip* gene were linked to arterial stiffness and coronary artery disease in a Brazilian population (Alvim et al, 2012). Also, differential expression of TXNIP variants in leukocytes is associated with acute myocardial infarction risk (Zhang et al, 2018). In clinical studies of coronary heart disease patients, *TXNIP* expression was detected to be higher in smokers than non-smokers (Mao et al, 2021); this could be related to ROS and inflammation-induced *TXNIP* expression.

TXNIP can control cardiac hypertrophy by regulating thioredoxin activity (Wang et al, 2002; Yoshioka et al, 2004), but it has dual roles on the heart. The absence of *txnip* was shown to not only protect the heart from ischemia injury in mice but also impair mitochondrial function (Yoshioka et al, 2012). The NLRP3 inflammasome-activating role of TXNIP may be particularly important for cardiac inflammation and cardiomyopathy (Liu et al, 2014; Peng et al, 2021; Wang et al, 2021a). TXNIP-NLRP3 activation in cardiac microvascular endothelial cells may participate in myocardial ischemia-reperfusion injury (Liu et al, 2014).

TXNIP was also recently discovered to participate in autophagy mechanisms. REDD1 is a negative regulator of mTOR, and TXNIP stabilizes REDD1 proteins, causing mTOR suppression (Jin et al, 2011). The REDD1-TXNIP complex was found to control stress-induced autophagy (Qiao et al, 2015). In recent studies, it was shown that TXNIP overexpression promoted cardiac autophagy and suppressed autophagosome clearance by increasing ROS in mice (Gao et al, 2020a).

### Skeletal Muscle

The molecular roles of TXNIP in different organs are also apparent in the insulin signaling pathway. The absence of TXNIP activates Akt in skeletal muscle tissues and the heart, but not in liver and adipose tissue (Hui et al, 2008). TXNIP was found to control both insulin-dependent and insulin-independent pathways of glucose uptake in skeletal muscles in human studies (Parikh et al, 2007). Physical exercise and TXNIP have a reciprocal relationship. Exercise reduces the protein levels of the TXNIP-REDD1 complex in skeletal muscle (Chaves et al, 2022). Mitochondrial fuel selection of skeletal muscle fibers is also controlled by TXNIP (DeBalsi et al, 2014). The absence of TXNIP results in significant shortages in amino acid, ketone, and lactate-break down enzymes in mice (DeBalsi et al, 2014).

### Liver

TXNIP is upregulated by both ChREBP and FOXO1 transcription factors in liver cells (Noblet et al, 2021). TXNIP KO mice were shown to be hypoglycemic and hypoinsulinemic, and they had diminished glucose production during a glucagon challenge, presenting a liver glucose homeostasis defect (Chutkow et al, 2008). *Txnip* null hepatocytes isolated from these mice could not produce sufficient glucose. Hepatic overexpression of TXNIP in wildtype mice caused increa-

sed serum glucose levels. Wildtype TXNIP protein rescued hepatocyte glucose production with a genetic *txnip* deletion, whereas the TXNIP-C247S mutant protein could not. TXNIP was also recently found to protect mice from fasting-induced liver steatosis through its effects on ER stress (Miyahara et al, 2022).

### Adipose Tissue

TXNIP is abundantly expressed in adipose tissues. TXNIP controls the intake of glucose into adipocytes by acting as an adaptor for the glucose transporter. Adipose tissues of TXNIP KO mice absorb more glucose than wildtype controls under starved conditions (Waldhart et al, 2017). Therefore, the deletion of TXNIP promotes adiposity and adipogenesis (Chutkow et al, 2010) and TXNIP degradation is required for adipocyte differentiation (Chutkow and Lee, 2011). On the other hand, the TXNIP-C247S mutation does not impair adipocyte differentiation, but TXNIP-C247S is more rapidly degraded. Interestingly, although adiposity is increased in these mice, insulin sensitivity is still preserved. Adipokines also regulate the TXNIP-inflammasome complex. Omentin-1 can limit adipose tissue inflammation in obese mice by repressing TXNIP-NLRP3 activation (Zhou et al, 2020).

### Pancreatic $\beta$ -Islets

As mentioned earlier, the absence of TXNIP in muscle and adipose tissue allows more glucose influx into these insulin-responsive tissues without insulin signaling, and liver-specific deletion of *txnip* inhibits gluconeogenesis (Chutkow et al, 2008). These characteristics effectively keep blood glucose low in TXNIP KO mice and make them less susceptible to insulin resistance. A deficit in functional  $\beta$  cells is a common identifying factor in both type 1 and type 2 diabetes (Thielen and Shalev, 2018). We know that TXNIP levels are positively regulated by glucose in  $\beta$  cells as in other cells; however, there are incomplete data on which glucose transporter interacts with TXNIP in  $\beta$  cells.

Transcriptomic analyses identified *txnip* as the most upregulated gene in glucose-treated human  $\beta$  cells (Richards et al, 2017), and TXNIP expression is elevated in  $\beta$  cells during diabetes (Minn et al, 2005; Xu et al, 2013). TXNIP methylation in whole-blood DNA was associated with hemoglobin HbA1c levels, type 2 diabetes, and metabolic syndrome in human populations (Soriano-Tárraga et al, 2015; Wang et al, 2021c; Yamazaki et al, 2022; Zhang et al, 2020). In a recent study, type 1 diabetes patients had significantly lower serum vitamin D and serum TXNIP levels (Omar et al, 2018).

In addition, TXNIP is subject to O-GlcNAcylation in response to high glucose concentrations, and O-GlcNAcylation of TXNIP appeared to be elevated in pancreatic islets of diabetic rats (Filhoulaud et al, 2019). However, glucose-induced *TXNIP* expression is counteracted by the activation of insulin receptors through histone deacetylases and by palmitate, which reduces oxidative stress in pancreatic  $\beta$  cells (Panse et al, 2018).

Higher TXNIP levels induced by either high glucose or ER stress are associated with  $\beta$  cell death (Chen et al, 2008b). Culturing of INS-1  $\beta$  cells and primary mouse and human islets with a glucose medium resulted in increased apoptosis levels (Shalev, 2008). TXNIP expression is induced by ER

stress by PERK/IRE1 pathways and results in IL-1 $\beta$  transcription through NLRP3 inflammasome signaling to trigger  $\beta$  cell death (Oslowski et al, 2012). In addition, TXNIP was found to affect the function of  $\beta$  cells; TXNIP induces miR-204 by inhibiting the activity of signal transducer and activator of transcription 3 (STAT3) in  $\beta$  cells to inhibit *mAF BZIP transcription factor A (MafA)*-mediated insulin transcription (Xu et al, 2013). TXNIP KO mice show higher total pancreatic insulin content and  $\beta$  cell mass than wildtype mice (Masson et al, 2009).  $\beta$  cell-specific *txnip* deletion increased  $\beta$  cell mass through Akt/B cell lymphoma-extra large (Bcl-xL) signaling and protected against diabetes in mouse models (Chen et al, 2008a).

### Kidney

TXNIP plays a significant role during nephropathy. High glucose levels increase TXNIP protein expression that triggers inflammatory and fibrotic responses in kidneys (Huang et al, 2016). In diabetic wildtype mice, renal injury and dysfunction indicators such as albuminuria and serum creatinine levels were increased, unlike in TXNIP KO mice (Shah et al, 2015). TXNIP also drives hyperglycemia-induced ROS generation by mitochondria in renal mesangial cells (Shah et al, 2013). Renal inflammation due to ureteral obstruction was suppressed in TXNIP KO mice, identifying TXNIP as a potential target to slow the development of chronic kidney diseases (Wu et al, 2018).

MicroRNAs can be triggering for the TXNIP-NLRP3 response. Exosomal microRNA miR-93-5p was found to target TXNIP-NLRP3 signaling to influence sepsis-induced acute kidney injury (Juan et al, 2021). Ablation of *txnip* was also shown to cause renal tubular lipid accumulation during diabetes by hindering the SREBP cleavage activating protein (SCAP) signaling pathway, yielding reduced cholesterol uptake and synthesis (Sun et al, 2021).

### Lung

Hypoxia-Inducible factor (HIF)-1 is a protein complex that plays a crucial role in the hypoxia response (Ziello et al, 2007). TXNIP can inhibit HIF-1-mediated gene transcription in the murine lung (Farrell et al, 2010). Inflammation-associated lung diseases such as asthma are linked to TXNIP. Inflammatory nanoparticles that aggravate asthma symptoms were shown to induce inflammation *via* TXNIP upregulation in mice (Lim et al, 2021). The extracellular signal-regulated protein kinase (ERK) pathway can control the pro-inflammatory cytokine tumor necrosis factor (TNF)-alpha-directed TXNIP degradation in respiratory epithelial cells (Kelleher et al, 2019), and inhibition of this pathway could reduce ROS in lung epithelial cells. Ubiquitination and degradation of TXNIP by ERK were dependent on the Thr349-phosphorylation of TXNIP inside the C-terminal domain, identifying this specific amino acid as a potential target for E3 ubiquitin ligases (Kelleher et al, 2019).

### Eye

TXNIP may play an important role in the retina. Viral gene delivery of *txnip* was found to ameliorate experimental Retinitis Pigmentosa in mice, which occurs due to the dysfunction or loss of cone-photoreceptor (Xue et al, 2021).

Gene delivery of *txnip* prolonged the survival of cone photoreceptors and improved visual acuity in mice, and this saved the cones by improving their lactate metabolism. Interestingly, gene delivery of TXNIP C247S yielded even more improvement. The authors hypothesized that by losing the TXNIP-thioredoxin interaction, both TXNIP and thioredoxin might be more beneficial (Xue et al, 2021).

### TXNIP and Cancer

TXNIP is associated with multiple cancer types and cancer cell malignancy (Meylan et al, 2021). TXNIP communicates with multiple fundamental intracellular pathways to act as a tumor suppressor. In a mouse strain that naturally had low TXNIP levels, hepatic carcinomas were common (Sheth et al, 2006). Lower TXNIP expression controlled by peroxisome proliferator-activated receptor gamma (PPARgamma) was found to be associated with advanced melanoma and metastases in patients with melanoma (Meylan et al, 2021). Further, in studies of malignant glioma, elevated TXNIP protein expression levels were associated with prolonged patient survival time (Zhang et al, 2017).

One of the cancer types where TXNIP plays a clear role through several molecular pathways is breast cancer. A decrease of TXNIP results in elevated proliferative activity and estrogen-induced cell growth in breast cancer (Park et al, 2018). A knockdown of the *txnip* gene in breast cancer cells was associated with glucose transporter 1 (GLUT1) induction and caused a substantial reduction in the cell-growth inhibitory effect of anti-estrogen agents. MicroRNAs were shown to be important in triggering metastasis *via* by TXNIP-HIF-1 signaling axis in breast cancer (Chen et al, 2015). TXNIP expression was discovered to be significantly correlated with HIF-1 expression in non-small cell lung cancer progression as well (Li et al, 2015).

Circular RNAs also suppressed tumor metastasis and glycolysis in lung adenocarcinoma by stabilizing TXNIP expression (Liang et al, 2021). A high expression of *c-myelocytomatosis* oncogene (*C-MYC*) and low expression of the *txnip* gene correlated with decreased survival in breast cancer (Shen et al, 2015). The MondoA-TXNIP axis is crucial for defining regulatory T cell identity and function in the progression of colorectal cancer (Lu et al, 2021). The effect of TXNIP on glucose uptake is also apparent in prostate cancer. Overexpression of TXNIP in prostate cancer cell lines inhibited glucose uptake, and TXNIP expression was lower in the human prostate cancer samples in a recent study (Xie et al, 2020).

TXNIP is also involved in kidney and liver cancers. Analysis of clinical data from The Cancer Genome Atlas Database identified TXNIP expression as a potential prognostic marker for patients with clear cell renal cell carcinoma (Gao et al, 2020b). Hepatitis B is known to cause hepatocellular carcinoma. Animal studies pointed out that TXNIP deficiency was sufficient to initiate hepatocellular cell carcinoma, and a truncated version of a Hepatitis B virus protein can cause hepatocarcinogenesis by downregulating TXNIP expression and reprogramming glucose metabolism (Sheth et al, 2006; Zhang et al, 2021).

In pancreatic cancer, TXNIP plays a different role. TXNIP protein and messenger RNA (mRNA) expression levels were higher in pancreatic cancer patients with diabetes, and

TXNIP-Thioredoxin-ROS axis was found to be regulated *via* p38 MAPK and ERK pathways in these tumors (Li et al, 2014). Several MicroRNAs also control TXNIP-HIF-1 $\alpha$  axis to control pancreatic cancer cell proliferation (Wang et al, 2021b; Zhu et al, 2018). Therefore, TXNIP has various significant roles in controlling tumor growth, and expression of TXNIP in cancer can sometimes be a predictor of cancer progression (Chen et al, 2020).

### TXNIP and Vitamin D Related and Other Diseases

TXNIP was initially named as “Vitamin D3 upregulated protein 1” due to increased levels by vitamin D(3) administration (Chen and DeLuca, 1994). Therefore, TXNIP might play a role in vitamin D-associated diseases. For example, TXNIP expression in serum and bones was increased in rat models of glucocorticoid-induced osteoporosis (Mo et al, 2021). TXNIP was also suggested to mediate the negative effects of glucocorticoid on osteoblast function and osteoclastogenesis in glucocorticoid-treated Cushing Syndrome patients with secondary osteoporosis (Lekva et al, 2012).

TXNIP expression in Multiple Sclerosis patients was significantly lower and serum thioredoxin levels were higher than the controls in a recent study (Mahmoudian et al, 2017). Genetic variations in the *TXNIP* gene were also associated with hypertension in a Brazilian population (Alvim et al, 2012). *TXNIP* and *Txn1* are differentially expressed in newly diagnosed multiple sclerosis patients or patients getting immunosuppressive treatments (Mahmoudian et al, 2017). miR-20a that plays a role in rheumatoid arthritis development may also play a part in the TXNIP-NLRP3-inflammasome pathway (Li et al, 2016).

### Conclusions

Here, we described multiple roles of TXNIP, including important roles in metabolism. TXNIP directly facilitates glucose transporter GLUT1-4 endocytosis. The evolution of C247 in TXNIP suggests a unique capability of TXNIP as a redox sensor connecting metabolite flux and redox flux. Further investigation of TXNIP function should contain both metabolic and redox components, so we can have a more thorough interpretation of phenotypes. The nuclear function of TXNIP is one important area of future studies.

### Authors' Contributions

All authors prepared and edited the article.

### Author Disclosure Statement

No competing financial interests exist.

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#### Abbreviations Used

ALDH1	= aldehyde dehydrogenase 1 family member 1
AMPK	= AMP-activated protein kinase
ARRDC	= arrestin domain-containing
BAT	= brown adipose tissue
cAMP	= cyclic AMP
ChREBP	= carbohydrate response element binding protein
ER	= endoplasmic reticulum
ERK	= extracellular signal-regulated protein kinase
FOXO	= forkhead box O
G6PD	= glucose-6-phosphate dehydrogenase
GLUT	= glucose transporter
GPCR	= G-protein coupled receptor
GSH	= glutathione
GSSG	= glutathione disulfide
H6PD	= hexose-6-phosphate dehydrogenase/ glucose 1-dehydrogenase
HIF	= hypoxia-inducible factor
IDH1/2	= isocitrate dehydrogenase [NADP(+)]
IL	= interleukin
IRE1	= inositol-requiring enzyme 1
KO	= knockout
ME	= malic enzyme
MTHFD1/1L	= methylenetetrahydrofolate dehydrogenase cyclohydrolase and formyltetrahydrofolate synthetase 1
mTOR	= mammalian target of rapamycin
NADPH	= nicotinamide adenine dinucleotide phosphate
NFkB	= nuclear factor kappa B
NLRP3	= NLR family pyrin domain containing 3
NNT	= nicotinamide nucleotide transhydrogenase
NRF2	= nuclear factor erythroid 2-related factor 2
OXPHOS	= oxidative phosphorylation
PERK	= PRKR-like endoplasmic reticulum kinase
PGD	= phosphogluconate dehydrogenase
PI3K	= phosphoinositide 3-kinases
REDD1	= regulated in development and DNA damage responses 1
ROS	= reactive oxygen species
slc2	= solute carrier family 2
TBP-2	= thioredoxin binding protein-2
Txn	= thioredoxin
TXNIP	= thioredoxin interacting protein
UPR	= unfolded protein response