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ABOUT COVER

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RESPONSIBLE EDITORS FOR THIS ISSUE

Thioredoxin interacting protein, a key molecular switch between oxidative stress and sterile inflammation in cellular response

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Abstract

Tissue and systemic inflammation have been the main culprit behind the cellular response to multiple insults and maintaining homeostasis. Obesity is an independent disease state that has been reported as a common risk factor for multiple metabolic and microvascular diseases including nonalcoholic fatty liver disease (NAFLD), retinopathy, critical limb ischemia, and impaired angiogenesis. Sterile inflammation driven by high-fat diet, increased formation of reactive oxygen species, alteration of intracellular calcium level and associated release of inflammatory mediators, are the main common underlying forces in the pathophysiology of NAFLD, ischemic retinopathy, stroke, and aging brain. This work aims to examine the contribution of the pro-oxidative and pro-inflammatory thioredoxin interacting protein (TXNIP) to the expression and activation of NLRP3-inflammasome resulting in initiation or exacerbation of sterile inflammation in these disease states. Finally, the potential for TXNIP as a therapeutic target and whether TXNIP expression can be modulated using natural antioxidants or repurposing other drugs will be discussed.

Key Words: Thioredoxin interacting protein; NOD-like receptor pyrin domain containing 3; Inflammasome; Interleukin 1b; Inflammation; Obesity; High-fat diet; Ischemia; Reperfusion; Oxidative stress

Core Tip: Inflammation has been postulated as the central pathway involved in maintaining homeostasis and in cellular response to insults. High fat diet-induced inflammasome activation have been reported to predispose microvascular diseases including retinopathy, and nonalcoholic fatty liver disease. Inflammation can alter vascular recovery in response to ischemic insult including ischemic retinopathy, stroke and critical limb ischemia. Thioredoxin interacting protein (TXNIP) is required for the activation but not necessarily for expression of NOD-like receptor pyrin domain containing 3-inflammasome resulting in initiation or exacerbation of the disease state. A list of natural antioxidants or repurposed drugs is included to modulate TXNIP expression.

INTRODUCTION

Sterile inflammation as a physiological and pathological response

Inflammation is the body's natural defense mechanism to recognize and react to harmful insults or stimuli in effort to eliminate or mitigate these damaging threats and maintain normal tissue and organ homeostasis[1,2]. Therefore, as illustrated in Figure 1, depending on the nature of these threatening insults or stimuli, inflammation can be generally classified into two major categories: (1) Microbial inflammation, resulting from the major group of microbial triggers, known as the pathogen associated molecular patterns (PAMPs). Examples of PAMPs include danger signals from invading microorganisms like; the whole microorganism (bacteria, virus or fungi), their byproducts (bacterial enzymes and/or toxins), or their subcellular components [bacterial lipopolysaccharide (LPS)]; and (2) Sterile inflammation, which is associated with non-microbial related insults, known as the damage associated molecular patterns (DAMPs). DAMPs include any chemical, biochemical triggers or metabolic by products or danger signals released as a result of tissue damage or cellular injury excluding microorganisms. Examples of DAMPs associated diseases include amyloid beta plaques in Alzheimer's disease (AD), cholesterol crystals in atherosclerosis, glucose in diabetes mellitus, glutamate in neurotoxicity and neurode-generative diseases, monosodium urate crystals in gout, and saturated fatty acids (ex: Palmitate) in obesity (Reviewed in[2-4]). Therefore, sterile inflammation can be defined as: Inflammation that occurs in absence of or irrespective to invading microorganisms or their byproducts. Both PAMPs and DAMPs triggers are recognized by a large family of pattern recognition receptors (PRRs) which provoke the expression of pro-inflammatory cytokines to further instigate the activation and recruitment of the pro-inflammatory and immune response *via* immune and non-immune cells. As illustrated in Figure 2, PRRs are generally classified into five major classes according to their subcellular location, activating PAMPs or DAMPs and their corresponding pro-inflammatory signaling pathways. NOD-like receptor (NLR), present in the cytoplasm, is one of the five major receptor classes of PRR that has been directly linked to major metabolic, micro and macrovascular diseases[2]. Upon recognition by a stimulus, NLR pyrin domain containing 3 (NLRP3) inflammasome signaling is initiated. NLRP3-inflammasome consists of the sensor NLRP3, the adaptor apoptosis-associated speck-like protein containing a CARD (ASC), and the effector caspase-1. NLRP3 activation process occurs *via* two steps: Priming and activation. The priming step requires NFkB-mediated transcriptional expression of the component of NLRP3-inflammasome and pro-cytokine namely pro-interleukin (IL)-1b. In contrast, pro-IL-18 is constitutively expressed. The assembly and activation of inflammasome result in pro-caspase-1 activation, which could subsequently cleave pro-cytokines namely pro-IL-1b and pro-IL-18 into their active forms IL-1b and IL-18, respectively[2,5].
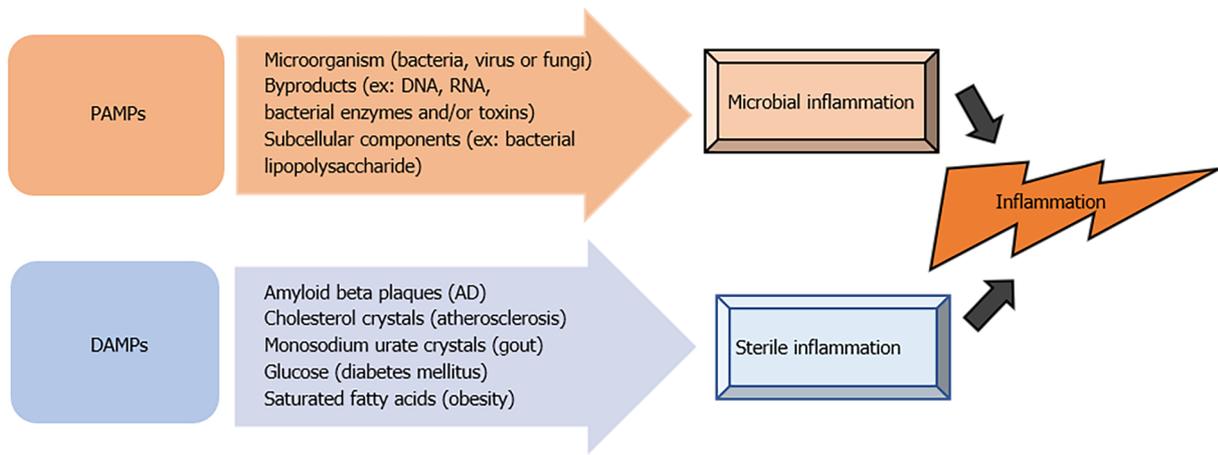
Figure 1 A diagram showing sterile inflammation and microbial inflammation. Inflammation results from activation of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). Examples of PAMPs include microorganisms, its byproducts or subcellular components. Examples of DAMPs include glucose, saturated fatty acids, uric acid, or amyloid beta plaques. DAMP: Damage-associated molecular pattern; PAMP: Pathogen-associated molecular pattern; AD: Alzheimer's disease.
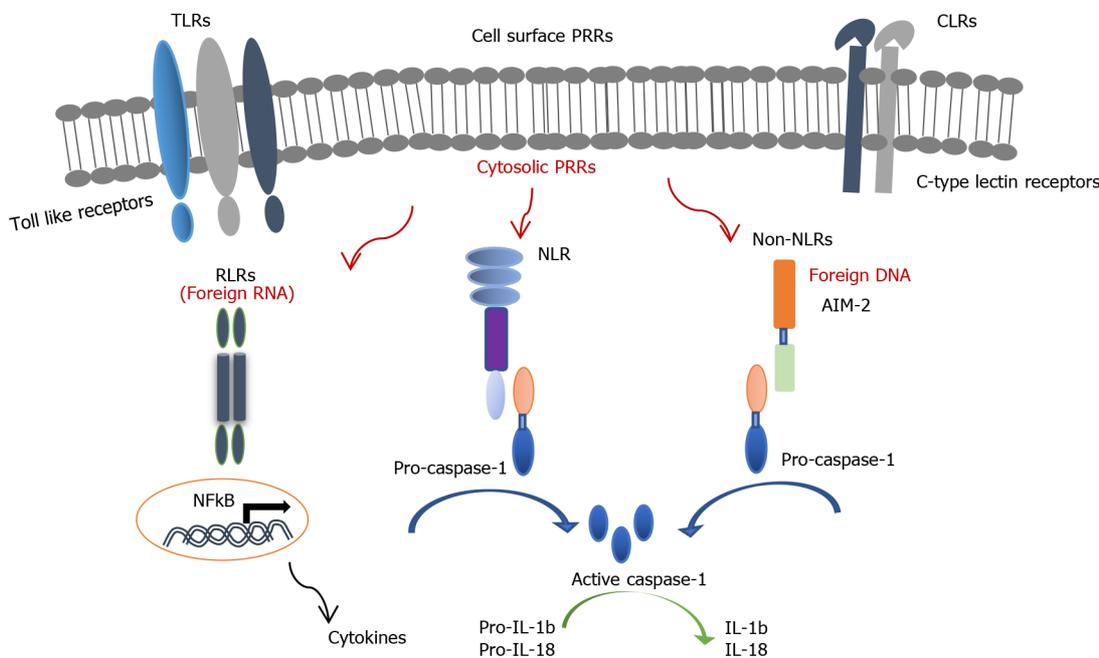
Figure 2 A diagram that depicts the five major classes of protein recognition receptors that are identified for sensing pathogen-associated molecular patterns and damage-associated molecular patterns and subsequent stimulation of proinflammatory responses. The cell surface pattern recognition receptors (PRRs) include Toll-like receptors and c-type lectin receptors. The cytoplasmic PRRs include NOD-like receptors (NLRs), retinoic acid-inducible gene-1-like receptors (RLRs), and the non-NLRs. RLRs recognize double-stranded RNA viruses and activate NFκB to increase the transcription of cytokines. The signaling of NLRs requires the initial expression of inflammasome and cytokine precursors such as pro-IL-1β or pro-IL-18. Assembly of the NLR-inflammasome results in caspase-1 activation and subsequently processing and secretion of cytokines IL-1β and IL-18. Non-NLRs, known also as AIM-2 can recognize double-stranded DNA viruses. Similarly, AIM-2 signals *via* activation of cleavage and release of active caspase-1 to process and mature IL-1β and IL-18. TLRs: Toll-like receptors; NLRs: NOD-like receptors; CLRs: c-type lectin receptors; PRR: Pattern recognition receptors; RLRs: Retinoic acid-inducible gene-1-like receptors.

TXNIP-NLRP3 inflammasome axis as a pivotal pathway for sterile inflammation

Thioredoxin-interacting protein (TXNIP), also known as vitamin D3 upregulated protein-1 or thioredoxin-binding protein-2, is an endogenous inhibitor of thioredoxin (TRX). TXNIP is a key regulator of oxidative stress and has been linked also to inflammation[2]. TRX serves as a multifunctional regulator that is necessary for the control of cellular survival, inflammation, glucose and lipid metabolism, angiogenesis, carcinogenesis, and oxidative stress[6]. Trx also acts on the apoptosis signal-regulating kinase 1 (ASK-1) to block the subsequent activation of the c-Jun N-terminal kinase (JNK) and p38 MAPK pathways that eventually leads to apoptosis stimulated by tumor necrosis

factor alpha (TNF-α)[7,8]. In the reduction-oxidation (redox) dependent state, the oxidized TXNIP-cysteine-247 binds to the active site of reduced TRX-cysteine-32 *via* disulfide bonds[6].

In addition, TXNIP works in a redox-independent fashion by stimulating NLRP3 inflammasome axis resulting in further activation of pro-caspase-1 and subsequently pro-IL-1β to IL-1β. As such, TXNIP is postulated to play a central role as a pro-inflammatory switch of the TXNIP-NLRP3 inflammasome axis. Several instigating metabolic insults and/or pro-inflammatory DAMPs converge to promote TXNIP-sterile inflammation as TXNIP is the common denominator for these metabolic stressors. This review will summarize the published evidence of TXNIP contribution in stimulating NLRP3-inflammasome and mediating sterile inflammation in response to metabolic and ischemic cellular events. In particular, we will attempt to highlight the major cellular activities that can trigger TXNIP and NLRP3 inflammasome activation and understand how sterile inflammation associated with high fat diet (HFD)-associated obesity and its impact on retinopathy, steatohepatitis and delayed vascular recovery. Further, we examined the impact of ischemia-reperfusion and the associated sterile inflammation in various disease states including ischemic retinopathy, ischemic stroke, brain aging and critical limb ischemia.

CELLULAR ACTIVITIES THAT CAN TRIGGER TXNIP AND NLRP3 INFLAMMASOME ACTIVATION

At the cellular level, sterile inflammation and activation of NLRP3-inflammasome could be triggered by multiple activities including K^+ efflux and lysosomal disruption induced by particles such as silica, cholesterol, and uric acid crystals. Mitochondrial dysfunction and formation of reactive oxygen species (ROS) are also important upstream events of NLRP3 activation. As depicted in Figure 3, TXNIP expression can be triggered at the transcription level by saturated fatty acids[9], hyperglycemia[10], calcium influx[7]. TXNIP can be post-transcriptionally regulated by endoplasmic reticulum (ER) stress and microRNA (miRNA)[11,12]. Further, TXNIP is an established regulator of cellular oxidative stress where ROS dissociates TXNIP from TRX and increase its level. Together, increases in TXNIP facilitate its binding to NLRP3, resulting in NLRP3-inflammasome activation (reviewed in[2]).

ER stress regulates TXNIP expression
Unfolded protein response (UPR) is an adaptive response, which prevents the accumulation of misfolded proteins in the lumen of the ER. The UPR is transduced by three major ER-resident stress sensors, namely protein kinase RNA-like ER kinase, activating transcription factor 6, and inositol requiring enzyme 1 (IRE1). When protein misfolding exceeds the capacity of the UPR, an ER-stress state that can trigger programmed cell death[13]. We and others have shown that ER stress can enhance TXNIP expression and NLRP3-inflammation, suggesting TXNIP as critical signaling node that links ER stress and inflammation[11,14,15].

Several studies have established the role of the Toll-like receptors (TLR) as upstream signal to mediate ER-stress response to the saturated fatty acid palmitate in cultured hepatocytes through the IRE1 pathway[16-18]. A prior study showed that TLR2 deficiency protected against hepatic steatosis in a murine model of diet-induced metabolic syndrome[17]. Deletion of TLR4 resulted in protection against development of non-alcoholic steatohepatitis (NASH) in apolipoprotein E deficient mice fed with high-fat and high-cholesterol diet as a model of obesity[18]. Release of glutamate, one of the well-identified DAMPs, was shown to trigger TXNIP expression[7,19,20]. Increased glutamate also has been shown to induce ER stress and result in neuronal damage in a model of brain ischemia[20]. Inhibition of ER stress with tauroursodeoxycholic acid reduced TXNIP activation suggesting the role of ER stress in the induction of TXNIP expression[20]. Ding *et al*[21] demonstrated a temporal relationship between TXNIP, ER stress and neuroinflammation in rat model of cerebral venous sinus thrombosis[21]. They found that oxidative stress and ER stress contribute to the activation of TXNIP, which further induces NLRP3 inflammasome and neuronal pyroptosis. Similarly, supplementation of ketogenic diet improved ischemic tolerance in mice through inhibition of ER stress and associated TXNIP-NLRP3 inflammasome activation[22].
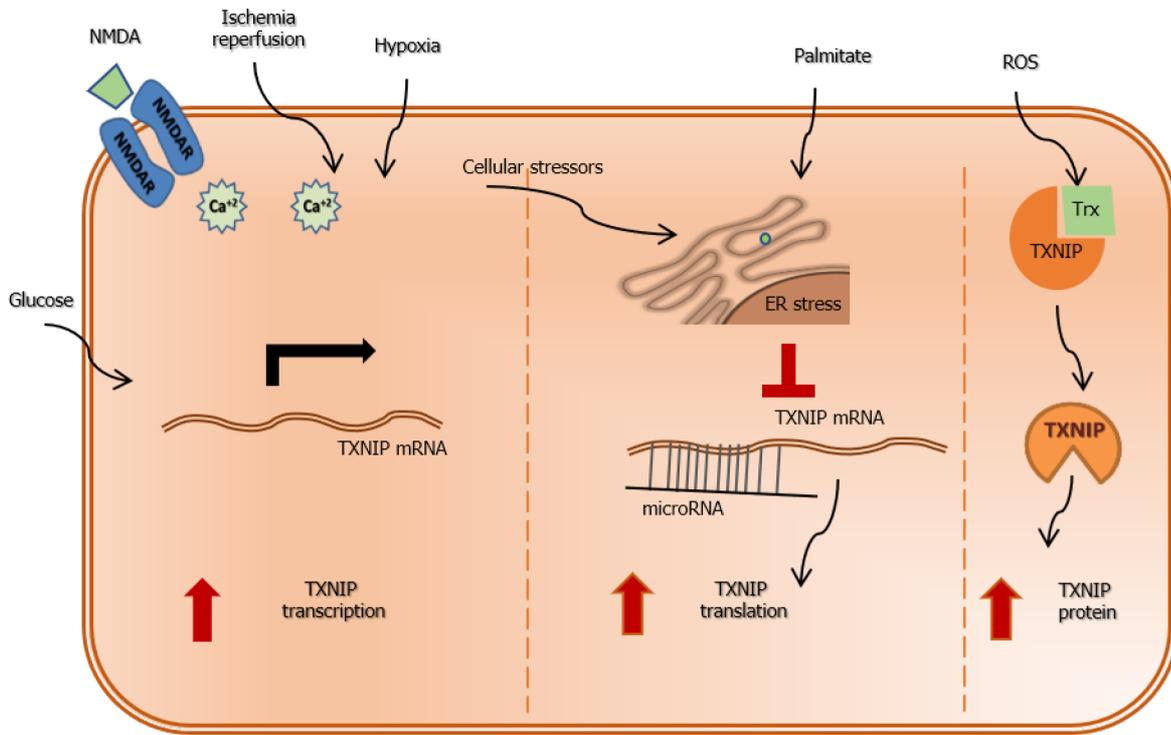
Figure 3 A diagram that depicts various ways of regulation of thioredoxin interacting protein expression. At the transcriptional level, thioredoxin interacting protein (TXNIP) expression can be triggered by hyperglycemia as it contains carbohydrate response element. Ischemia-reperfusion injury, hypoxia and activation of the n-methyl D-aspartate receptor result in significant increase in calcium influx that trigger TXNIP expression *via* activation of the Ca-response element. Further, TXNIP can be post-transcriptionally regulated by endoplasmic reticulum (ER) stress and microRNA (miRNA) that traditionally bind to the 3 UTR region of TXNIP mRNA and repress its translation. Under conditions of cellular stressors, saturated fatty acids such as palmitate result in increases in ER stress and degradation of miRNA resulting in increases in TXNIP expression. Finally, oxidative stress and formation of reactive oxygen species dissociates TXNIP from thioredoxin and increase its level that facilitate activation of NLRP3-inflammsome and release of inflammatory mediators. TXNIP: Thioredoxin interacting protein; NMDA: N-methyl D-aspartate; ER: Endoplasmic reticulum; ROS: Reactive oxygen species.

Role of miRNA and regulation of TXNIP expression

miRNAs are a class of highly conserved, endogenous non-coding RNAs containing 19 to 25 nucleotides. miRNA anneal to target genes and simultaneously control their translation and transcription. The stability of mRNA is governed by binding of specific miRNAs to complementary sequences in the 3' untranslated region (UTR) of gene target, which is then degraded or silenced[23]. Bioinformatic analysis of the TXNIP 3' UTR identified several conserved binding sites for miRNA that were implicated in the negative regulation of TXNIP expression (reviewed in[13]). Recent literature shows growing number of newly-identified miRNA that regulate TXNIP expression in various models. One of the examples include miR-20b-3p that has been demonstrated to negatively regulate TXNIP expression in models of diabetic retinopathy and cerebral ischemia[24,25]. Another example is miR-146a-5p, that has been shown to regulate TXNIP expression and subsequently the inflammatory and apoptotic response in human chondrocytes cell line[26]. TXNIP was also reported to be a potential target of miR-125b promoting metastasis and progression of pancreatic cancer *via* the HIF1α pathway[27,28]. In colorectal cancer tissues, miR-135b-5p was upregulated whereas TXNIP was downregulated, which promoted cell proliferation, migration and invasion, and suppressed apoptosis of cancer cells[29]. Overexpression of miR-148a reduced infarct size in vivo, and alleviated dysregulation of cardiac enzymes and Ca²⁺ overload in myocardial ischemia/reperfusion *via* down-regulating TXNIP and inactivating the TLR4/NF-κB/NLRP3 inflammasome signaling pathway [30]. Myocardial ischemia reperfusion induced TXNIP expression and lower miR-150-5p levels, along with increased cardiomyocyte apoptosis[31]. Injection of MSCs-derived exosomes containing miR-150-5p resulted in downregulation of TXNIP and showed a reduction in myocardial remodeling[31]. Similarly, myocardial I/R triggered TXNIP expression both mRNA and protein in diabetic mice and miR-135a expression level was reduced in diabetic mice regardless of I/R injury or not[32]. Of note, the regulation of TXNIP expression by miR-17-5p is well-studied in various models and will be discussed in the following section.

Role of miRNA miR-17-5p in regulation of TXNIP expression

Recent work showed that known DAMPs such as palmitate and hypoxia triggered the expression of ER-stress markers and TXNIP in retinal Müller cells[11,33]. Among the ER-stress markers, IRE1α, is an ER bifunctional kinase/RNase that has been shown to destabilize number of RNA and miRNA including miR-17-5p. The latter is a small non-coding RNAs that control the translation and transcription of TXNIP[12]. When levels of miR-17-5p decline under stress condition, TXNIP expression is enhanced. Our work demonstrated that treatment of retinal Müller cells with ER-stress inhibitor phenyl-butyric acid or with the pharmacological inhibitor of IRE1α, dramatically restored TXNIP expression back to normal level[11]. Similarly, exposure to strong DAMPs such as hypoxia *in vitro* and ischemic/reperfusion injury *in vivo* has been shown to trigger ER-stress and TXNIP expression[33,34]. Modulation of miR-17-5p activity or expression reduced the increased TXNIP-NLRP3-mediated inflammation [33,34]. These studies highlight the potential contribution of ER stress to fine-tune expression of TXNIP and regulate its associated inflammation. In consistence, several reports have shown that ER stress can enhance TXNIP expression and NLRP3-inflammation, suggesting TXNIP as critical signaling hub that links ER stress and inflammation[11,14,15].

Cross-talk of miR17-5p, PPAR-g and TXNIP expression

Umbelliferone, a natural antioxidant, has been proven effective against neurodegenerative and inflammatory processes[35]. Inhibition of TXNIP expression and NLRP3 inflammasome activation was also associated with upregulation of PPAR-γ. Interestingly, maintaining the redox state suggest a balance between activation of PPAR-γ and down-regulation of TXNIP, indicating that PPAR-γ is a negative regulator of TXNIP[36]. In particular, TXNIP expression has been demonstrated to be regulated by PPAR isoform PPAR-β/δ. Treatment with GW0742, PPAR-β/δ, agonist attenuated the expression of TXNIP-NLRP3, microglial activation and improved neurological outcome in rat pups following hypoxic ischemia[34]. The inhibitory effect of PPAR-β /δ on TXNIP is also mediated by upregulation of transcriptional regulator miR17-5p confirming the regulatory role of PPARs on TXNIP[37].

OBESITY AND HFD ARE MAJOR DRIVERS FOR STERILE INFLAMMATION

Obesity is characterized by excessive accumulation of fat in the body that impairs personal health and a body mass index of more than 30. Among all the detrimental causes of obesity, HFD and lack of exercise are the most compelling factors[1]. Because of the accumulation of macronutrients in the adipose tissue, inflammatory mediators, such as TNFα and IL-6, are stimulated, which lead to the activation of pro-inflammatory state and oxidative stress[1]. Further, chronic sterile inflammation is not only integral to the pathobiology of obesity, but it also causes development of insulin resistance resulting in a vicious cycle to sustain obesity[38]. obesity is a risk factor for development of type II diabetes, cardiovascular events and can cause microvascular dysfunctions[39]. Therefore, understanding the interaction between obesity and sterile inflammation in various disease states has become crucial. In the next section, we will examine the role of TXNIP in mediating the interaction between obesity and sterile inflammation in select disease state including retinopathy, steatohepatitis and delayed vascular recovery after critical limb ischemia.

TXNIP-NLRP3 inflammasome activation and HFD-systemic inflammation

Similar to humans, when rodents are fed with HFD, they present with an increase of body weight, total cholesterol, triglyceride, insulin resistance and high glucose level[9, 40,41]. We and others showed that HFD is associated with increased systemic and local production of IL-1β detected in the circulation and across several tissues[40,42]. The contribution of TXNIP is best demonstrated by the findings that genetic deletion of TXNIP not only resulted in alleviated glucose intolerance in HFD, but it also mitigated HFD-induced systemic and tissue inflammation[9,40].

Direct role of TXNIP-NLRP3 Inflammasome activation in microvascular dysfunction

Microvascular dysfunction and subsequent cell death can be generally classified according to the root cause into: (1) Primary dysfunction; that occurs as a result of mechanical, metabolic and/or biochemical insults and associated sterile inflammation within the vascular cells themselves (ex: Endothelial cells, pericytes); and (2)

Secondary dysfunction; that occurs in response to the sterile inflammation driven by the same kind of insults in non-vascular cells (ex: Infiltrating and resident proinflammatory monocytes and macrophages and/or specialized cells like astrocytes and glial cells). In response to different types of DAMPs, several studies have established the direct role of TXNIP-NLRP3 Inflammasome activation in several microvascular beds. Ischemia reperfusion injury was shown to induce the specific TXNIP-mediated upregulation and activation of the NLRP3 inflammasome axis and associated oxidative stress, endothelial barrier dysfunction and pro-inflammatory response in animal models of cerebral and cardiac ischemia and corresponding cultured brain and cardiac microvascular endothelial cells rather than non-vascular cells[43,44]. Furthermore, similar findings were also reported showcasing the protective effect of genetic and pharmacological inhibition of the TXNIP-NLRP3 inflammasome axis and associated microvascular dysfunction, vascular permeability, apoptosis, and angiogenic response in hyperglycemic animal models of diabetic retinopathy and cultured retinal endothelial cells[24,45,46].

In effort to dissect the direct role of the TXNIP-NLRP3 inflammasome axis in microvascular *vs* non-vascular cells, cultured retinal endothelial cells treated with saturated fatty acid palmitate coupled to BSA to mimic HFD-obesity showed significant increase in TXNIP expression as well as NLRP3-inflammasome activation and IL-1β expression[9,11,41]. Knocking-down TXNIP expression mitigated expression and activation of NLRP3-inflammasome components evident by increased caspase-1, and IL-1β[9,11]. Furthermore, overexpression of TXNIP plasmid mimicked the HFD-induced sterile inflammation *in vitro* evidenced by the significant elevation of NLRP3, caspase-1, IL-1β, and TNF-α, which were attenuated upon treatment with IL-1 receptor antagonist. Hence, indicating the essential role of the TXNIP-NLRP3 inflammasome activation in driving sterile inflammation in autocrine fashion through IL1-b[41]. Similarly, isolated Muller cells from TXNIP knockout (TKO) mice showed a blunted NLRP3 inflammasome activation response to saturated fatty acid palmitate treatment compared to the primary cultures from WT mice[11]. Interestingly, our studies in cultured retinal endothelial cells showed that knocking down TXNIP expression specifically blunted palmitate-induced, but not peroxynitrite-induced release of IL-1b to the condition medium[9]. TXNIP, as a member of the alpha arrestin scaffolding proteins, plays an essential role in intracellular cargo trafficking. Hence, subcellular localization of TXNIP in response to different insults might enhance or inhibit release of mature IL-1β[9]. These findings confirmed the integral role of TXNIP in not only expression and activation of IL-1β, but also its release extracellularly and to the systemic circulation.

TXNIP-NLRP3 inflammasome activation and HFD-induced retinal microvascular dysfunction and degeneration

In addition to the central role of TXNIP in mediating HFD-induced metabolic response, TXNIP can directly connect HFD-induced metabolic stress and sterile inflammation. Clinical and preclinical studies have established obesity among other components of the metabolic syndrome as an independent risk factor risk for development of retinal microvascular dysfunction with or without diabetes[47-51]. Our group was the first to report that HFD can selectively result in inducing TXNIP expression and its direct association with NLRP3 inflammasome activation evidenced by increased cleaved caspase-1 and cleaved IL-1b levels in a rat model of HFD for 8-10 wk[9]. In this study, immunohistochemical analysis revealed strong TXNIP expression in the retinal ganglion cell layer and inner nuclear layer, which colocalized within Müller cell end-feet and retinal microvasculature that constitute two major components of the retinal neurovascular unit[9]. In subsequent series of studies, we further elucidated that HFD triggers the unfolded protein ER-stress response in retinas from 4 wk HFD fed mice *in vivo* and in cultured retinal Muller cells treated with saturated fatty acid palmitate. Inhibiting ER-stress significantly blunted the increase in HFD-induced TXNIP expression without altering its associated insulin resistance in the HFD treated group. In line with our findings, another report also showed that retinas from HFD exhibited neural inflammasome activation at 3-mo of HFD, before the development of systemic glucose intolerance, electroretinographic defects, or microvascular disease[52]. Moreover, HFD or Western diet was also shown to enhance TXNIP expression in the retina that resulted in TXNIP-dependent JNK activation and retinal cell death[53]. Retinal neurodegeneration and decreased retinal function were also observed in response to HFD combined with Streptozotocin injection as a model for type 2 diabetes, that was alleviated by over expression of TRX suggesting the involvement of the TXNIP-mediated activation of the mTOR pathway and associated

inhibition of autophagy[54]. Furthermore, we sought to examine the effect of TXNIP deletion on HFD-induced retinal microvascular inflammation and degeneration. In WT mice, short-term HFD feeding for 8 wk resulted in enhanced TXNIP expression along with increased levels of cleaved caspase-1, cleaved IL-1b as the classic markers for NLRP3 inflammasome activation. Increased levels of retinal adhesion molecules, ICAM-1 and VCAM-1, retinal vascular permeability and associated leukocytes-induced obstruction of the retinal vasculatures, were also elevated[9,41]. However, such effects were ameliorated in TXNIP knock out mice fed with HFD. In parallel, 18 wk of HFD also resulted in increased degeneration of retinal microvascular capillaries and morphological changes in WT mice fed; on the other hand, TXNIP deletion abrogated such presentations[9,41]. Together, these findings signify the direct causal role of TXNIP in mediating HFD-Induced NLRP3 inflammasome activation and retinal microvascular dysfunction.

TXNIP-NLRP3 inflammasome activation and HFD-induced steatohepatitis

The global prevalence of non-alcoholic fatty liver disease (NAFLD) is 25.2% with a 40.7% incidence rate of developing into cirrhosis, which is a sequala resulted from NASH, a serious form of NAFLD [systemic review by Younossi *et al*[55]. Among individuals with NAFLD, 51.94% of them have a comorbidity of obesity, 22.5% with type-II diabetes, 69.2% with hyperlipidemia, and 42.5% metabolic syndrome, which implicated the strong correlation between steatohepatitis and metabolic dysfunctions [55]. While the pathogenesis of disease progression from NAFLD to NASH is not fully understood, involvement of sterile inflammation, TLR signaling, and gut-liver axis are among suggested pathways[56].

Several studies established the role of the TLRs and downstream activation of the NLRP3 inflammasome in the pathophysiology of NAFLD using various models of diet-induced liver dysfunction[17,57-59]. HFD-induced hepatic steatosis was associated with statistically significant expression of TLR2, total-NFκB, NLRP3, cleaved caspase-1, cleaved IL-1β, and TNF-α[58]. Genetic deletion of TXNIP attenuated the expression of TLR2, T-NFkB NLRP3 and their downstream inflammatory markers [58]. In the same study, IL-1β expression coincided within the same areas of steatosis, inflammatory cell infiltration, collagen deposition and α-SMA expression in WT-HFD mice[58]. We and others have shown that genetic deletion of TXNIP resulted in the alleviation of hepatic steatosis, hepatocyte inflammation, and fibrogenesis[17,57,58]. In parallel, genetic modulation or pharmacological inhibition of caspase-1, as a central mediator, protected against HFD-induced hepatic steatosis, inflammation and early fibrogenesis[60-62]. In addition, both genetic deletion and pharmacological inhibition of NLRP3 reduced hepatic inflammation and the expression of hepatic caspase-1 and IL-1β to normal level in two murine models of steatohepatitis fed by methionine/choline deficient or atherogenic-HFD diet models[57,63]. Interestingly, knocking-down TXNIP augmented steatohepatitis and hepatic fibrosis in methionine choline-deficient diet-fed mice[64]. Such discrepancy could be attributed to the different nature of experimental model of steatohepatitis.

Multiple studies using the pharmacological inhibition of TXNIP and NLRP3-inflammasome showed reduction in the hepatic pro-inflammatory markers and lipid accumulation by using salidroside, verapamil, quercetin, allopurinol, dietary curcumin, salvianolic acid A, and berberine[21,65-67]. Mitigating oxidative stress and TXNIP expression in the liver resulted in suppression of TNF-α, NLRP3, caspase-1, and IL-1β in a mouse model of HFD-induced NAFLD or from the diabetic mouse model induced by either streptozotocin injection[66] or fructose-fed diet[68]. Kim *et al* [69] depicted that dietary curcumin could also downregulate TXNIP expression, which protected fatty liver in a high fat/high sugar induced mouse model. Furthermore, berberine also demonstrated its ameliorative effect on steatohepatitis in methionine-choline deficient-fed mice; and its inhibitory effect of NLRP3 inflammasome activation *via* the ROS/TXNIP axis *in vitro*[70].

ISCHEMIA-REPERFUSION AS A MAJOR DRIVER FOR STERILE INFLAMMATION

Post-ischemic event, the restoration of blood flow is usually the primary therapeutic approach as reperfusion is essential to restore oxygen and nutrients. However, reperfusion can induce further tissue damage in the ischemic organ and adjacent ones. Ischemia-reperfusion is associated with alteration of intracellular calcium level and release of ROS, known upstream triggers of cell injury. ROS disturb TRX system by

increasing TXNIP expression and inhibiting TRX from binding and inhibiting the activity of ASK-1, resulting in activation of apoptotic pathways. In parallel, displacement of TXNIP triggers NLRP3-inflammasome activation and subsequent inflammation signaling pathways. Further, TXNIP has been shown to modulate angiogenic response in addition to metabolic and inflammatory response[71]. Prior study showed that TXNIP is required for VEGF-mediated angiogenic signal and response in endothelial cells[72]. In the next section, we will review the evidence of how TXNIP contributed to NLRP-3 inflammasome activation in the pathogenesis of ischemic-reperfusion and secondary microvascular dysfunction and/or neurodegenerative events.

TXNIP-NLRP3 inflammasome axis activation in critical limb ischemia

Critical limb ischemia is a major symptomatic manifestation of peripheral arterial disease and failure to establish revascularization eventually leads to amputation. Traditionally, TRX stimulates ischemia-induced angiogenesis through two primary mechanisms; by regulating ROS that resulted in increased level of nitric oxide and nitrotyrosine formation; or by suppressing the ASK-1 that promotes endothelial cells apoptosis[73]. Recent evidence shows compromised functional recovery to ischemia in patients with frank diabetes and in non-diabetic patients with insulin resistance.[74] We and others have shown that metabolic disorders including obesity is associated with impaired vascular recovery post-ischemic events in experimental models[40,75, 76]. Increases in oxidative stress and TXNIP expression have been implicated as major players in impaired vascular recovery. Indeed, deletion of TXNIP improved vascular recovery and protected mice from the reduction of blood flow due in HFD-obesity[40, 77]. One of the possible mechanisms of impaired vascular recovery is the loss of ischemia-induced VEGF expression and/or activation of its receptor (VEGFR2) due to metabolic stress. Interestingly, the expression of VEGF and VEGFR2 activation were similar across mice lacking TXNIP expression (TKO) regardless of the diet choice (normal diet *vs* HFD) or ischemic condition (ischemic side *vs* sham)[40]. The other postulated mechanism of impaired vascular recovery is the activation of NLRP3-inflammasome in response to stress conditions such as ischemia or HFD, which led to a rise in inflammatory mediators such as IL-1β[40,77]. Deletion of TXNIP significantly reduced NLRP3-inflalmmasome activation evident by reduced tissue and circulatory level of IL-1b[40]. In agreement, genetic deletion of TL4 or NLRP3 demonstrated similar vascular protective effects *via* modulation of TXNIP-NLRP3-inflammasome activation[76,78]. These studies confirmed the integral role of TLR4-mediated TXNIP and NLRP3-inflammasome signaling interfering with perfusion recovery in muscle ischemia. These signaling molecules may represent a therapeutic target to improve vascular recovery and preserve limb salvage.

TXNIP-NLRP3 inflammasome activation in retinal ischemia-reperfusion model

Retinal neurodegeneration, an early characteristic of several blinding diseases, triggers glial activation, resulting in inflammation, secondary damage and visual impairment. Treatments that focused only at neuroprotection have failed clinically. Therefore, there is significant need for treatment strategy that target secondary damage. Exposure to transient ischemia alters TRX antioxidant defenses, resulting in significant increases in retinal oxidative stress and TXNIP expression. Indeed, exposure to transient ischemia resulted in early increase in TXNIP mRNA expression that persisted for 14 d in a model IR, compared to sham controls[33]. Colocalization studies showed that TXNIP localized within activated glial Müller cells in IR-retinas. Exposure of Müller cells to hypoxia-reoxygenation injury triggered ER stress markers and inflammasome activation in cells isolated from WT mice, but not in cells isolated from TKO mice. Secondary damage was triggered by TXNIP-NLRP3-inflammasome activation evident by increase in inflammatory mediators, sustained neurodegeneration. Furthermore, secondary damage was sustained 14-days post IR injury assessed by the significant increase in the number of occluded acellular capillaries and visual impairment in IR-WT mice, but not in IR-TKO. Intervention with TXNIP-antisense oligomers (ASO) prevented ischemia-induced glial activation and neuro-vascular degeneration, and improved visual function compared to untreated WT[33]. In a recent study, sulforaphane administration significantly inhibited IR-mediated changes in retinal thickness and prevented retinal ganglion cell death. Sulforaphane suppressed inflammatory cytokines production, microglia activation, and inflammasome activation. In parallel, knockdown of NLRP3 was performed, and the according changes of retinal ganglion cells assessed. In accordance, NLRP3 knockdown presented the similar inhibitory effect on IR rats[79]. The protective of the effect of TXNIP inhibition is partly mediated through inhibition of NLRP3-inflammasome components including cleaved

caspase-1 and IL-1b[80]. Therefore, targeting TXNIP expression may offer an effective approach in the prevention of secondary damage associated with retinal neurodegenerative diseases.

TXNIP-NLRP3 inflammasome activation in ischemic stroke

Ischemic stroke is a leading cause of death and long-term disability in the US with limited therapeutic window for reperfusion therapy. Thus, there is a great need to identify effective therapeutics that could be administered in a more practical window. Recent mRNA profiling analysis by Tian *et al*[81] demonstrated that TXNIP signaling is one of the major gene hubs differentially expressed in rat brain following middle cerebral artery occlusion. In agreement, our studies showed that ischemic injury-induced TXNIP expression was associated with significant increases in expression of NLRP3-inflammasome components and its activation[80]. Consistently, genetic deletion or pharmacological inhibition of TXNIP with resveratrol resulted in protection of mice from ischemic reperfusion injury and improved neurological outcome following embolic middle cerebral artery occlusion[80]. Elevated expression of TNF-α, and apoptotic markers including cleaved caspase-3 and PARP were attenuated by TXNIP deletion or resveratrol treatment. We and others demonstrated also that genetic deletion of TXNIP or overexpression of TRX have showed neuroprotective effects against ischemic brain damage[80,82]. TKO mice showed higher expression of TRX with reciprocal decrease in the makers of oxidative stress including nitrotyrosine along with inhibition of inflammation activation. In support, Hua *et al* [83] demonstrated in rat model with middle cerebral artery occlusion/ reperfusion, increased expression TXNIP, elevated level of markers of oxidative stress and reciprocal decrease in the expression TRX.

Diabetes is the leading co-morbidity, which increases the risk of hemorrhagic transformation and poor recovery in stroke patients. Recently, we reported that thrombolytic therapy with tissue plasminogen activator (tPA) worsened ischemic reperfusion injury under hyperglycemic condition along with activation TXNIP-NLRP3 axis[84]. Pharmacological modulation of TXNIP expression with verapamil attenuated NLRP3 inflammasome activation, hemorrhagic transformation, and blood brain barrier (BBB) damage. Treatment with verapamil attenuated activation of ASC, cleaved caspase-1 and IL-1β[84]. Although verapamil is not a specific activator for TXNIP, it can be considered as an adjunctive therapy to mitigate the detrimental effect of tPA in hyperglycemic condition[85]. Consistently, attenuation of TXNIP-NLRP3 activation with hyperbaric oxygen preconditioning also activation thereby ameliorated hyperglycemia associated hemorrhagic transformation and stroke outcome[3]. In support, Cao *et al*[43] demonstrated that ischemic stroke associated BBB damage along with activation of TXNIP and NLRP3 inflammasome and activation of MAPKs including p38 and JNK. Further, treatment with ruscogenin, an anti-inflammatory steroid sapogenin could inhibit the activation of MAPK, TXNIP/NLRP3 pathway and BBB damage in mouse model of ischemic stroke. Ruscogenin attenuated the ischemia associated activation of NLRP3-inflammasome and subsequently mitigated levels of caspase-1 and IL-1β.

TXNIP-NLRP3 INFLAMMASOME ACTIVATION IN NEURODEGENERATION

TXNIP-NLRP3 inflammasome and development of AD

Neurodegenerative diseases such as AD, Parkinson's, and Huntington's disease are characterized by the progressive loss of specific synapses and neurons[86]. Microglia are the principle innate immune cells in the CNS that express TLRs, and it has been shown that amyloid plaques, characteristic of AD can activate the innate immune response by interacting with TLRs[87]. Excessive inflammation can be linked to altered TLR4 signaling and increased possibility of developing AD[87,88]. HFD has been implicated in driving inflammation in regards to AD. It has been shown that a caloric deficit has the ability to possess anti-inflammatory effects and downregulate the TLR4/NF-$_κ$B signaling cascade[89]. Several studies have shown the protective effects of TXNIP inhibition against NLRP3-inflammasome activation in genetic models of cognitive dysfunction and AD. Examples of these models include the Swedish mutant form of APP (APPswe)/PSEN1dE9 transgenic mice[90], ibotenic acid-induced neurological disorder in rats and in cells[87].

TXNIP-NLRP3-inflammsome activation in brain aging

Recent study by Oberacker *et al*[91] demonstrated that TXNIP is candidate gene for aging as is found to be elevated in aged human primary T cells, hematopoietic progenitor cells and monocytes. Consistently, elevated mRNA expression of TXNIP was reported in aged human cortices (81-95 year) in comparison with young (25-37 year)[92]. Later, Zhou *et al*[67] found that aging is associated with elevated microglial activation and neuroinflammation along with increased TXNIP/NLRP3 expression in aged rat (24 mo) in comparison with young rats (12 mo). Further, modulating TXNIP-NLRP3 activation with a traditional anti-inflammatory Chinese medicine attenuated age-associated neurodegeneration in aging rats[93]. Consistently, we recently identified aged-dependent activation of TXNIP-NLRP3 inflammasome in the cortex and hippocampus of brain in both male and female mice[94]. Our studies showed that enhanced TXNIP expression in aged mice is associated with decreased TRX expression and oxidative damage. It is further evident from increased expression of ASC, cleaved caspase-1 and cleaved IL-1β. Genetic deletion of TXNIP attenuated activation NLRP3-inflammasome activation with parallel decrease in the expression of caspase-1 and IL-β. Further, pharmacological inhibition of TXNIP with verapamil significantly attenuated neuroinflammation and age associated-cognitive impairment confirming the contributory role of TXNIP in age associated neurodegeneration[94].

THE POTENTIAL FOR TXNIP AS A THERAPEUTIC TARGET

TXNIP is a multi-faceted protein that plays pro-inflammatory, pro-oxidative stress, and pro-apoptotic functions. As reviewed, TXNIP mediated NLRP3-inflammasome activation has been implicated in the detrimental micro- and macrovascular complications of various disease states. Although TXNIP has been widely suggested a promising therapeutic target, there is lack of specific pharmacological inhibitor. Therefore, other therapeutic tools for inhibiting TXNIP were developed that extend from natural compounds, phytochemicals to repurposing of drugs that are already approved for clinical use[71,95]. In the next section, we will review some of the potential therapeutic tools for targeting TXNIP expression.

Natural antioxidants inhibit TXNIP expression

Pharmacologically, there are number of natural antioxidants that have been reported to exert their protective effects *via* inhibition of TXNIP and NLRP3-inflammasome (see Table 1). Such list included the following; salidroside, quercetin, allopurinol, dietary curcumin, salvianolic acid A, and berberine[21,65,66]. The protective effects of these natural antioxidants were mainly mediated by mitigating oxidative stress, TXNIP and NLRP3 expression and lipid accumulation in the liver, which led to future suppression of TNF-α, NLRP3, caspase-1, and IL-1β in a mouse model of HFD-induced NAFLD.

Ischemic stroke models were used to demonstrate the protective effects of natural and herbal medicine *via* inhibitory effect on TXNIP expression. Taohong Siwu decoction (THSWD), a traditional Chinese medicine could inhibit the expression of TXNIP thereby improved neurobehavioral outcome, inhibited NLRP3-inflammasome activation and pyroptosis following ischemic reperfusion injury in rat[96]. Treatment with THSWD attenuated activation of NLRP3-inflammasome evident by lower level of caspase-1, IL-1β, TNFα, IL-6, HMGB1 and IL-18. Similarly Z-Guggulsterone, a herbal steroid has shown its protective effect in ischemic reperfusion injury by inhibiting TXNIP/NLRP3-inflammasome demonstrated by down regulation of IL-1β, IL-6, and IL-18[97]. Wang *et al*[98], demonstrated that Umbelliferone, a natural antioxidant belongs to coumarin derivative, could inhibit TXNIP expression in a rat model of focal cerebral ischemia. Pretreatment with Umbelliferone, 7-d before ischemic stroke ameliorated infarct size and brain edema with improved neurological outcome[98]. The beneficial effects of Umbelliferone is mediated by inhibition of TXNIP-NLRP3 inflammasome activation[35]. Treatment with GW0742, PPARβ/δ, agonist attenuated the expression of TXNIP-NLRP3, microglial activation and improved neurological outcome in rat pups following hypoxic ischemia[34].

Drug repurposing to inhibit TXNIP expression

Repurposing of clinically approved drugs in the market provide promising and safe therapeutic options (listed in Table 2). Verapamil, a calcium channel blocker, has been widely used to inhibit TXNIP expression. Verapamil-mediated TXNIP inhibition is conferred by reduction of intracellular calcium, inhibition of calcineurin signaling, and nuclear exclusion. Verapamil has been shown to exert antidiabetic effects and reduce

Table 1 Summary of studies on modulation of thioredoxin interacting protein using natural antioxidants animal models

Ref.	Treatment	Animal model	Main findings
[1]	Taohong Siwu decoction 18, 9 and 4.5mg/kg Intragastric administration for 7 d	Rat with middle cerebral artery occlusion	Improved neubehavioral function and inflammation and inhibited pyroptosis following ischemic stroke
[2]	Z-Guggulsterone, 12.5, 25, 50 mg/kg, (*ip*) Intraperitoneal administration for 6 d	Rat with middle cerebral artery occlusion	Z-Guggulsterone improved neurological deficit and, modulated redox imbalance and inflammation through inhibition of TXNIP/NLRP3 signaling
[11]	Curcumin 50 mg/kg, One hour before surgery, (*ip*)	Rat with cerebral artery occlusion	Attenuated ischemic brain injury. Modulation of TXNIP/NLRP3 inflammasome activation by suppression of ER stress.
[70] [69]	Curcumin	HFD/ High sugar diet	Prevented fatty liver *via* inhibition of TXNIP
[66]	Qurecetin	diabetes	Prevented inflammation, liver TXNIP, lipid accumulation
[6]	Ketogenic diet 3 wk	Mouse model of middle cerebral artery occlusion	Ketogenic diet improved ischemic tolerance, Attenuated ER stress and TXNIP/NLRP3 activation
[7]	Umbelliferone, 15and 30 mg /kg Pretreatment for 7 d (*ip*)	Rat with middle cerebral artery occlusion	Protected against cerebral ischemia reperfusion injury by suppressing TXNIP/NLRP3 inflammasome activation
[8]	Ruscogenin, 10 mg/kg One hour before surgery, (Intra gastic admin.)	Mice with middle cerebral artery occlusion	Decreased brain infarction, edema, improved neurological outcome by suppressing a TXNIP/NLRP3 inflammasome activation and MAPK pathway
[9]	Resveratrol, 5 mg/Kg 3 h post-embolic occlusion. (*iv*)	WT mice with embolic middle cerebral artery occlusion	Protected from ischemic injury, improved neurological score suppressed TXNIP/NLRP3 inflammasome and apoptosis
[21]	Salvianolic acid	HFD- Rats	Prevented HFD-induced NAFLD
[65]	Salidroside		Prevented HFD-induced NAFLD
[12]	Compound 10b, 3 mg/kg At the onset of reperfusion	Rat with middle cerebral artery occlusion	Attenuated cerebral ischemia by upregulating endogenous antioxidant system and down regulation of oxidative stress.

TXNIP: Thioredoxin interacting protein; NLRP3: NOD-like receptor pyrin domain containing 3; HFD: High fat diet.

glucose toxicity *via* decreasing the binding of carbohydrate response element-binding protein to the E-box repeat in the TXNIP promoter[10]. Oral administration of verapamil prevented N-methyl D-aspartate (NMDA)-induced retinal neurotoxicity by three different mechanisms, inducing release of inflammatory mediators such as TNF-α and IL-1β, altering antioxidant status and disrupting the Trx-ASK-1 inhibitory complex leading to activation of the p38 MAPK/JNK apoptotic pathway[7]. Inhibiting TXNIP expression with verapamil attenuated NLRP3 inflammasome activation, hemorrhagic transformation, BBB damage[84]. Verapamil improves hepatic inflammation and improves metabolic homeostasis in NAFLD[67].

Other drugs such as metformin has been shown to reduce TXNIP expression *in vitro* using differentiated macrophages in response to high glucose[99]. In vivo, treatment of apoE$^{-/-}$ mice alleviated diabetes-induced metabolic disorders and atherosclerosis. The postulated protective mechanism of metformin involved inhibition of TXNIP-mediated NLRP3 inflammasome activation[99]. Earlier study using STZ-diabetes model showed that metformin and resveratrol can modulate ROS production and ER-stress *via* reducing TXNIP expression. Further, metformin mitigated inflammation and apoptosis *via* inhibition of TXNIP and NLRP3-inflammasome activation[100].

Finally, Ezetimibe, hypolipidemic drug has shown its beneficial effect in ischemic stroke by modulation of TXNIP-NLRP3 inflammasome activation through modulation of AMPK and nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway [101]. Activation of Nrf2 with tert-butylhydroquinone inhibited the activation TXNIP/NLRP3-inflammasome in a rat model of MCAO[102]. Conversely, genetic deletion of Nrf2 abolished the activation of TXNIP following MCAO demonstrating the contribution of oxidative stress in the activation of TXNIP in ischemic stroke.

Table 2 Summary of studies on modulation of thioredoxin interacting protein expression using drug repurposing in animal models

Ref.	Treatment		Animal model	Main findings
[84]	Verapamil (0.15 mg/kg), intra venous	1 h	Hyperglycemic mouse model middle cerebral artery occlusion	Reduced infarct area, hemorrhagic transformation and blood brain barrier damage. Improved stroke outcome and neuro inflammation in response to hyperglycemic stroke
[7]	Verapamil po	1 h	NMDA- optic neuropathy	Improved retinal neurodegeneration by altering antioxidant status and disrupting the Trx-ASK-1 inhibitory complex
[67]	Verapamil, 25 mg/kg/d, IP	1 wk	high-fat diet-induced obesity- 10 wk	Improved hepatic inflammation, metabolic homeostasis in NAFLD *via* TXNIP-NLRP3 inflammasome activation
[104]	Verapamil		High-fat diet-prediabetic neuropathy	improved prediabetic neuropathy, inflammation *via* inhibition of TXNIP and NLRP3-inflammasome activation
[10], [105]	Verapamil, 100 mg/kg	Po daily	STZ- and HFD-obesity model	Inhibit TXNIP expression and restore beta-cell function, improve glucose level in STZ- and HFD-obesity model
[100]	Metformin		STZ-diabetes mouse	Suppressed TXNIP/NLRP3 inflammasome activation, reduced cell apoptosis in adipose tissue
[99]	Metformin		ApoE$^{-/-}$ + STZ mice	Inhibited TXNIP/NLRP3 inflammasome activation, and suppressed diabetes-accelerated atherosclerosis in apoE-/- mice
[101]	Ezetimibe (250 µg, 500 µg, 1 mg)	1 hIntra-nasal	Rat model middle cerebral artery occlusion	Improved infarct volume, neurological outcome Increased activation of AMPK, modulated oxidative stress, microglial activation and TXNIP/NLRP3 activation
[103]	SRI-37330	Po daily	STZ-mouse model and obesity-induced (db/db) diabetes	Inhibited glucagon secretion and function, reduced hepatic glucose production, and reversed hepatic steatosis
[105]	W2476, 200 mg/kg	Po daily	STZ- and HFD-obesity model	Inhibit TXNIP expression and restore beta-cell function, improve glucose level in STZ- and HFD-obesity model
[34]	GW0742 (25 µg/kg; intranasal)	1 h/ 24 h	Rat pups with hypoxic ischemia	GW0742 significantly reduced the activation of TXNIP/NLRP3 inflammasome, pro-inflammatory microglia

TXNIP: Thioredoxin interacting protein; NLRP3: NOD-like receptor pyrin domain containing 3; HFD: High fat diet; NMDA: N-methyl D-aspartate.

Since TXNIP modulates several of the essential metabolic and homeostatic pathways, there are challenges that can hinder the full development of TXNIP as a druggable target[71,95]. For instance, ablation of TXNIP at the systemic level carries a considerable risk for disrupting the physiological roles of TXNIP in regulating beta cell function, insulin release and regulating fatty acids metabolism[71,95]. Of note, targeting TXNIP may pose possible risks for sacrificing its proapoptotic action in the treatment of cancer[95], a disease state that is beyond the scope of this review. On the other hand, targeted delivery of specific TXNIP inhibitors in more confined organs (like the retina or the liver) might provide safer therapeutic opportunities. For example, intravitreal injection of TXNIP ASO prevented vision loss post-IR injury[33]. The protective effects of TXNIP ASO involved mitigation of TXNIP expression, inflammasome activation and secondary damage[33]. A novel oral form of small molecule SRI-37330 that shows benefit effects against the development of obesity and diabetes [103]. The drug has attenuated the formation of hepatic glucose and reversing steatohepatitis *via* the inhibition of TXNIP[103]. Finally, expanded pre-clinical studies for similar types of new drug molecules, followed by larger studies in subsequent stages of clinical development will remain key in ultimate evaluation of the efficacy *vs* the safety promise for TXNIP as a very attractive therapeutic target.

CONCLUSION

In summary, sterile inflammation is a central pathway that is involved in both physiological and pathological cellular processes to maintain homeostasis. TXNIP, a pro-oxidative, pro-inflammatory and pro-apoptotic protein that has been implicated in sterile inflammation. Evidence from literature showed that TXNIP is required for the activation but not necessarily expression of NLRP3-inflammasome in response to various stimuli as summarized in Table 3 for *in vivo* studies and Table 4 for *in vitro* studies. While there is no specific inhibitor for TXNIP, there is a long list of natural antioxidants and other drugs that could be repurposed are to modulate TXNIP expression (see Tables 1 and 2). Furthermore, potential specific small molecule

Table 3 Summary of the *in vivo* studies

Ref.	Duration of Studies	Insult	TXNIP	NLRP3	CASP-1	IL-1β	TNF-a	NFKB	Casp-3	NY	Other markers
Mohamed et al[2], 2015	Rat retina, 10 wk	HFD	+	+	+	+	+	+	+	+	Acellular capillaries
Coucha et al[11], 2017	Mouse retina, 8 wk	HFD	+ mRNA								ER-stress, miR17-5p
Mohamed et al[41], 2020	Mouse retina, 8 wk	HFD	+	-	-	+					Leukostasis, acellular capillaries
Mohamed et al[58], 2018	Mouse liver, 8 wk	HFD	+	+	+	+	Trend	+			TLR2 signal +, fibrosis
Elshaer et al[40], 2017	Mouse sk. Muscle, 8 wk	HFD	+	-	+	+				+	Systemic IL-1b, vascular recovery
Coucha et al[33], 2019	Mouse-retina, 1-3 d, 14 d	I/R	+ protein + mRNA	+	+	+	+				Acellular capillary, visual acuity
El-Azab et al[19], 2014	Mouse-retina, 1-d	NMDA	+			+	+		+	+	Acellular capillary, neurode-generation, ERG
Al-Gayyar et al[7], 2011	Rat-retina, 1-d	NMDA	+			+	+	+	+	+	Neurode-generation
Ishrat et al[80],2015	Mouse; Brain	Embolic-stroke	+	+	+	+	+		+	+	Neurological function, cerebral blood flow
Ismael et al[94], 2021	Mouse brain, 24 h	Stroke+ HG	+	=	+	+	+	+ trend			Hemorrhagic transformation
Wang et al[24], 2020	Rat brain, 7-d	Stroke	+	+	+	+		+			Pyroptosis, inflammation
Liu et al[97], 2020	Rat brain, 7 d	Stroke	+ mRNA + protein	+	-	+	+				Neurological deficit, inflamm
Gamdzyk et al[34], 2020	Rat pups brain, 24 h	Hyp-oxia	+	+	+	+					Microglial activation, TXNIP
Ding et al[21], 2016	Rat brain, 14 d	Throm-bosis	+	+	+	+				+	ER- stress neural pyroptosis
Yin et al[29], 2021	Rat brain, 72 h	Stroke	+	+	+	+					Microglial activation, ROS
Tian et al[81], 2012	Rat brain, 24 h	Stroke	+								MAPK activa-tion and Nrf2
Guo et al[3], 2018	Mice, 72 h	Stroke	+	+	+ active	+					Elevated ER stress, neurode-generation
Hou et al[102], 2018	Rat brain, 24 h	Stroke	+	+		+					Nrf2 and NL-RP3 through TXNIP

Ref.	Model	Insult	TXNIP	NLRP3	CASP-1	IL-1β	TNF-a	NFKB	Casp-3	Other markers
Cao et al [43], 2016	Mice brain, 24 h	Stroke	+	+		+				Neuro. deficit, BBB damage
Guo et al [3], 2016	Rat brain, 24 h	HG + stroke	+	+	+	+				Hemorrhagic transformation
Hua et al [83], 2015	Rat brain, 24 h	Stroke	+					+	+	Neurological deficit
Wang et al [98], 2015	Rat brain	Stroke	+	+	+	+				PPARγ, nega-tive regulator of TXNIP
Li et al [20], 2015	Rat brain, 24 h	Stroke	+	+	+	+				ER stress me-diates TXNIP activation

HFD: High fat diet; TXNIP: Thioredoxin interacting protein; NLRP3: NOD-like receptor pyrin domain containing 3; ROS: Reactive oxygen species; ER: Endoplasmic reticulum; BBB: Blood brain barrier.

Table 4 Summary of the *in vitro* Studies

Ref.	Cell type	Insult	TXNIP	NLRP3	CASP-1	IL-1β	TNF-a	NFKB	Casp-3	Other markers
Mohamed et al [2], 2015	EC	Palmitate	+	+	+	+			+	IL1-b in cell lysate and CM Adhesion Molecules
Mohamed et al [41], 2020	EC	TXNIP++		+	trend	+	+			Adhesion Molecules
Coucha et al [11], 2017	Muller	Palmitate	+ protein + mRNA	trend	trend	+				IL1-b in cell lysate
Coucha et al [33], 2019	Muller	Hypoxia	+ mRNA	trend	+	+				IL-1b in cell lysate
El-Azab et al [19], 2014		NMDA	+	+	+	+	+			IL1-b in CM
Gamdzyk et al [34], 2020	P12 cells	OGD	+			+	+			Cell death, miR-17-5p
Tian et al [81], 2012	Primary rat cortical neuron	OGD	+							Oxidative stress and activation of MAPK
Liu et al [97], 2020	Primary rat neurons	OGD	+	+	+	+				TXNIP NLRP3
Guo et al [3], 2018	SH-SY-5Y cells	OGD		+	+ activity	+				Activation of ER stress
Cao et al [43], 2016	bEnd.3	OGD	+	+	+					MAPK activation, EC-damage

TXNIP: Thioredoxin interacting protein; NLRP3: NOD-like receptor pyrin domain containing 3; ER: Endoplasmic reticulum; TNF-α: Tumor necrosis factor alpha; NMDA: N-methyl D-aspartate.

inhibitors are currently under development and will provide a much-needed treatment for TXNIP-associated disease states.

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