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Thioredoxin-Interacting Protein (TXNIP) Associated NLRP3 Inflammasome Activation in Human Alzheimer's Disease Brain

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Abstract

Alzheimer's disease (AD) is the most common form of age-associated dementia characterized by amyloid-β plaques and neurofibrillary tangles. Recent studies have demonstrated that thioredoxin-interacting protein (TXNIP), an endogenous regulator of redox/glucose induced stress and inflammation, is now known to be upregulated in stroke, traumatic brain injury, diabetes and AD. We hypothesized that TXNIP overexpression sustains neurodegeneration through activation of the nucleotide binding and oligomerization domain-like receptor protein 3 in human AD brains. We analyzed TXNIP and the components of the NLP3 inflammasome in the cortex of postmortem human brain samples by western blotting, real-time PCR, and immunohistochemical techniques in comparison with age-matched non-demented controls. Our results demonstrate that TXNIP protein as well as its mRNA levels in the cortex was significantly upregulated in AD compared to control brains. Moreover, using double immunofluorescence staining, TXNIP and interlukin-1β (IL-1β) were co-localized near Aβ plaques and p-tau. These results suggest an association between TXNIP overexpression levels and AD pathogenesis. Further, a significant increased expression of cleaved caspase-1 and IL-1β, the products of inflammasome activation, was detected in the cortex of AD brains. Together, these findings suggest that TXNIP, an upstream promising new therapeutic target, is a molecular link between inflammation and AD. The significant contribution of TXNIP to AD pathology suggests that strategies focusing on specific targeting of the TXNIP-NLRP3 inflammasome may lead to novel therapies for the management of AD and other age-related dementias.

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Keywords

Alzheimer's disease; amyloid plaques; hyperphosphorylated tau; NLRP3 inflammasome interlukin-1; thioredoxin-interacting protein

INTRODUCTION

Alzheimer's disease (AD) is the sixth leading cause of death in the United States, affecting over 5.7 million people [1]. AD is characterized by the extracellular buildup in amyloid-β (Aβ) plaques and the accumulation of intracellular neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau (*p*-tau) and accompanied by degeneration and dementia [2]. Although the precise molecular mechanisms underlying neurodegeneration in AD are complex and poorly understood, series of studies suggest that oxidative stress and inflammatory responses are a prominent and early feature in the pathogenesis of AD [3–5]. Despite considerable efforts to develop efficient compounds, no antioxidant therapy has successfully passed clinical trials. Regarding the complexity of the underlying pathophysiology, pleotropic targets may presumably provide desirable efficiency in AD patients. Thioredoxin-interacting protein (TXNIP) is an endogenous inhibitor of the thioredoxin (TRX) pathway, a major cellular thiol-reducing and antioxidant system [6]. Besides redox regulation, TXNIP is a key component in glucose metabolism, insulin resistance and inflammation [7–11], all of which are implicated in the pathogenesis of AD [4, 5, 12].

Recently, our group and others have demonstrated that TXNIP is required for the activation of the cytosolic pattern recognition receptor nucleotide-binding and oligomerization domain (NOD)-like receptor protein 3 (NLRP3) inflammasome [9, 13–16], a recently-discovered mechanism mediating sterile inflammation in several diseases including stroke, AD, and aging as well [5, 16–18]. Upon sensing several damaging signals including Aβ aggregates and oxidative stress, NLRP3 recruits the adapter protein apoptosis-associated speck-like (ASC) and pro-caspase-1, leading to caspase-1 production and subsequent interlukin-1β (IL-1β) maturation and release [19, 20]. The significance of NLRP3 inflammasome activity and IL-1β release is quite well-founded in AD pathology [21, 22]. In this connection, inhibiting NLRP3 signaling in AD mice has been shown to rescue cognitive impairment [23], reduce Aβ deposition, and improve memory function [24, 25]. IL-1β as the product of NLRP3 inflammasome oligomerization, is a key player in the innate immune response in AD, and experimental data have also revealed that blocking IL-1β signaling may prevent cognitive impairment in a mouse model of AD [23].

These evidences support a significant role for TXNIP as an upstream instigator of progressive degeneration in AD brains. Notably, accumulating evidence suggest that divergent damaging signals converge on TXNIP, either induced by damage-associated molecular patterns (DAMP; i.e., cellular debris or released ions) or oxidative stress. This in turn leads to essential biological effects including TRX repression and NLRP3 inflammasome activation. With strong effect to drive oxidative and inflammatory cascades to detrimentally activate endogenous kinases, TXNIP is an established mediator to impair

insulin functionality in the brain [26]. Alternatively, the redox sensitive TXNIP effector, phosphatase and tension homolog (PTEN) may reduce PIP3, which may turn off Akt function [27, 28]. These findings have encouraged recent researches to focus on TXNIP function in animal models of CNS diseases including AD. Accordingly, discernible TXNIP overexpression has been documented in the hippocampus and entorhinal cortex of the 5xFAD transgenic mouse model mice as an early AD phenotype [29–32].

As a step toward identifying new therapeutic targets for AD, the aim of this study is to determine whether human AD-linked changes are consistent with the preclinical findings on TXNIP. With supporting data indicating that the IL-1β is elevated in both the brain and plasma of AD patients [33], we hypothesize that TXNIP overexpression is associated with the NLRP3 inflammasome and IL-1β in human AD brains. There are no human data available yet on an association between TXNIP overexpression and pathological hallmarks of AD. The present study therefore, examines postmortem human brains of aged subjects diagnosed with AD and compares the relevant molecular findings with age-matched non-AD brains. Our findings are the first to demonstrate TXNIP overexpression and co-localization with Aβ and p-tau in human AD brains, providing empirical evidence for a close link between TXNIP and AD molecular pathology.

MATERIALS AND METHODS

Ethical approval

This study was approved by the UTHSC Institutional Review Board (IRB #17–05716-NHSR; Exempt Application 665258), Memphis, TN, USA, and was conducted under standard ethical procedures. All the appropriate personal protection safety procedures were followed to handle the human samples.

Human brain specimens

Table 1 demonstrates the characteristics of AD patients and the brain specimens were used in immunostaining or immunoblotting examinations. For western blotting, non-AD and AD patients' tissue specimens were obtained from the Human Brain and Spinal Fluid Resource Center, which is sponsored by NIHDS/NIMH, National Multiple Sclerosis Society, and the Department of Veterans Affairs. Samples were derived from short-postmortem interval (PMI) autopsies from the University of Kentucky AD Center (UK-ADC) cohort [34].

As addressed in our earlier report [35], the pre-mortem neuropathological assessments were performed using standard neuropathological procedures as described in detail elsewhere [36, 37]. Frontal cortical sections used for the western blotting analyses were snap-frozen during the autopsy in liquid nitrogen and stored at −80°C until the time of immunoblotting. The inclusion criteria included: PMI < 4 h; no clinical evidence of frontotemporal dementia or pathologic evidence of frontotemporal lobar degeneration; no cancer in the brain parenchyma and no large infarctions in the whole brain, or micro-infarcts found within 3 cm of the brain tissue samples.

For immunofluorescent staining, postmortem AD human brains with duration of dementia from 5 to 12 years and neuropathological criteria for definite AD were obtained from the

University of Iowa Deeded body program, Iowa City, IA, USA. The age-matched control brains (non-AD) were obtained at routine autopsy, from patients dying without any history of neurological or psychiatric illness. The tissues were collected within 3–8 h of death. The temporal lobe blocks were dissected and immersion-fixed in 4% paraformaldehyde solution. Blocks were cryoprotected with 30% sucrose until sunk, frozen sections were cut at 40 μm on a sliding microtome and these sections were collected in phosphate buffer saline (PBS) and stored in cryo storage solution (glycerol 30 ml, ethylene glycol 30 ml, 40 ml 0.1 M PBS) until being used for immunofluorescence staining.

Western blotting

Brain tissue samples were homogenized and processed for western blotting as previously described [38]. Thirty-μg of protein was loaded in each lane followed by transfer to PVDF membranes. The membranes were blocked for non-specific binding and probed with primary antibodies against TXNIP (1:1000; NBP1–5 4578, Novus Biologicals), TRX (1:1000; C63C6, Cell Signaling Technology), NLRP3, cleaved caspase-1, ASC (1:1000; AG-20B-0014; AG-20B-0042; AG-25B-0006, Adipogen Life Sciences), pro-IL-1β or cleaved IL-1β (1:1000; ab9722; Abcam, USA) at 4°C overnight. Following TBS-T washes, the membranes were incubated with horseradish peroxidase–conjugated secondary antibody (1:10,000, Sigma). The bands were then visualized by means of an enhanced chemiluminescent substrate system (Thermo Fisher Scientific). Protein levels were analyzed densitometrically using Image J software, normalized to loading controls, and expressed as fold change.

Real-time PCR

To estimate TXNIP mRNA transcripts, real-time PCR was carried out as described earlier [39]. Briefly, total RNA was extracted from AD and non-AD brain tissues using RNeasy® Mini Kit (Cat. #74104, Qiagen, Germany). Following the RNA isolation, genomic DNA was removed and single-strand complementary DNA was synthesized using QuantiNova™ Reverse Transcription Kit (Cat.#205 413, Qiagen, Germany). The oligonucleotide primer pair 5′-CAGCAGTGCAAACAGACTTCGG-3′ (forward) and 5′-CTGAGGAAGCTCAAAGCCGAAC-3′ (reverse) specific for human TXNIP were selected for qPCR amplification (Integrated DNA Technologies, IA). Real-time quantitative PCR was performed using PowerUp™ SYBR™ Green Master Mix (Applied Biosystems) and Bio-Rad CFX connect™ Real-Time system (Serial#BR003568, Bio-Rad Laboratories, CA). The thermal cycler parameters were as follows: UDG activation at 50°C for 2 min, and Dual-Lock™ DNA polymerase activation at 95°C for 2 min, followed by amplification of cDNA for 40 cycles with denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. The values were normalized using human β-actin mRNA level as an endogenous internal standard (specific oligonucleotide primer forward 5′-CACCATTGGCAATGAGCGGTTC-3′, reverse 5′-AGGTCTTTGCGGATGTCCACGT-3′, Integrated DNA Technologies, IA).

Immunofluorescence staining

Coronal sections of temporal cortex were washed with 0.1% PBS-TritonX-100 for 20 min. For most included antibodies, antigen retrieval was done using sodium citrate buffer

with 0.1% Triton-X. Sections were blocked with 5% BSA for 1 h at room temperature and were incubated at 4°C with either of the primary antibodies, including mouse Anti-Aβ (1:250; 9888S, Cell signaling technology), mouse anti-phospho-tau-Ser262 (1:250; 44–750G, Invitrogen), rabbit anti-cleaved-IL-1β (1:200), TXNIP (1:100; NBP1–5 4578, Novus Biologicals). The sections were then washed three times followed by incubation with the respective Alexa Fluor- 488 (042-03-18-06) and/or Alexa Fluor −594 (A-11012) tagged secondary antibodies (1:200) for 1 h at room temperature. Thereafter sections were washed and mounted with ProLong™ Diamond Antifade Mountant with DAPI (Invirogen), and viewed using a Zeiss 710 confocal laser scanning microscope. Negative controls were prepared by omitting the primary antibodies.

Statistical analysis

All statistical analysis was performed using the GraphPad Prism Instat 7.0 software by Student's *t*-test for two groups (AD and non-AD brains). Where a normality test could not be passed, Mann-Whitney test was used for comparisons. Quantitative data in all graphs were presented by means ± SD (standard deviation of mean). $p < 0.05$ was considered statistically significant.

RESULTS

Increased expression of TXNIP in the human AD brains

Western blot analysis carried out on frontal cortex extracts from AD and control brains showed significantly increased protein expression (around two-fold) in a molecular mass around 55 KD corresponding to TXNIP (Fig. 1A; $t(10) = 2.65$, $p < 0.05$). To determine if the enhanced protein expression is due to increased TXNIP transcription, we performed real-time PCR analysis on the cortical samples. Accordingly, TXNIP mRNA showed a high expression levels in the frontal cortex of AD patients compared to age-matched controls (Fig. 1B; $t(18) = 4.35$, $p = 0.0004$), indicating that TXNIP over-expression may occur at transcriptional level in AD brains. Immunostaining of postmortem AD brain sections also demonstrated a remarkable TXNIP overexpression in the temporal cortex. Importantly, double staining of AD cortices with TXNIP and molecular hallmarks of AD pathology showed that TXNIP is overexpressed in vicinity of Aβ plaques and p-tau as well (Fig. 1C) suggesting a pathological association between AD and TXNIP function. TXNIP overexpression in temporal cortices of AD patients were further confirmed with TXNIP intensity analysis in our stained specimens (Fig. 1D, $p = 0.028$).

Decreased expression of TRX in human AD brains

Given the established impact of TXNIP to directly affect TRX expression, we performed TRX immunoblotting in cortical samples obtained from postmortem brains. Based on densitometric analysis TRX expression was significantly reduced in AD brains (Fig. 2; $t(9) = 2.65$, $p < 0.05$). Coupled with high TXNIP levels, this concludes diminished TRX expression and might predict substantial insufficiency of TRX system and its antioxidant functionality in human AD brains.

Expression of NLRP3 inflammasome and the subsequent caspase-1 and IL-1β maturation in human AD brains

Frontal cortices were subjected to further immunoblotting to determine whether the detected TXNIP overexpression was accompanied by NLRP3 inflammasome activation to cleave pro-caspase-1 and drive IL-1β maturation (Fig. 3). Based on densitometric analysis a sharp upregulation was detected for both caspase-1 (($t(14) = -3.69$, $p < 0.01$) and cleaved-IL-1β ($t(13) = -2.80$, $p < 0.01$) in AD compared to non-AD brain samples supporting high cleavage activity of the NLRP3 inflammasome. ASC, an essential element for NLRP3 inflammasome oligomerization, also showed significant overexpression in AD cortices (Fig. 3C; $t(12) = 2.29$, $p < 0.05$). However, the NLRP3 inflammasome protein did not show any elevation in the immunoblots (Fig. 3B), the increase in caspase-1 cleavage and IL-1β maturation conclude that the existing NLRP3 inflammasomes in AD brains possess higher cleavage activities.

IL-1β upregulation and co-localization with Aβ plaques in the temporal cortices of human AD cortices

As a pivotal inflammatory cytokine, IL-1β is a strong effector of NLRP3 inflammasome system activity in microglial cells, driving AD tissue damage. Non-AD sections still showing fair amounts of IL-1β expression though, the higher IL-1β staining intensity in human AD temporal cortices confirmed the enhanced cytokine expression revealed in the immunoblot analysis (Fig. 4; $t(6) = -2.42$, $p < 0.05$). Interestingly, in the double immunofluorescent staining of temporal AD cortices we found that IL-1β is expressed in the vicinity of Aβ plaques. IL-1β over-production might indirectly indicate higher NLRP3 inflammasome activity, which in turn is under TXNIP influence. Consistent with the observed TXNIP co-localization with Aβ plaques, these data may support a role for TXNIP and NLRP3 inflammasome in AD pathology in aged human brain.

DISCUSSION

Information about exact pathological mechanisms driving AD mostly stems from quantitative transcript analysis in specimens acquired from patients with proven clinical manifestations. Nevertheless, spatial or temporal correlations are required to legitimately claim any causative link. Several proteome and microarray analysis have demonstrated sharp alterations in the so-called “proteinopathies” like AD. Genes regulating neurological functions [40], encoding inflammatory molecules [41, 42], or metabolic elements [43] show altered expression in AD brains. Besides precise tandem mass spectrophotometric analysis [44], recent advances in functional *in vivo* brain imaging have also provided strong evidence of spatial correlations between the expression of genes implicated in glucose metabolism and tau or Aβ pathologies in human brain [45]. This is supported with the earlier microarray study indicating mRNA transcription of genes involved in type 2 diabetes are obviously modified in either human or animals AD brains [46]. Accordingly, AEBP1, TXNIP, VCAM1, and ANGPT1 were upregulated in AD hippocampus while 16 others showed a remarkable decline. As demonstrated by our immunoblots and confirmed in immunostaining images, the myeloid intracellular protein TXNIP exists in control brain (non-AD) tissue where it has several biological roles in metabolism and oxidative stress. It mainly resides

within glial cells and may contributes to insulin resistance by mediating oxidative damage to insulin receptors and reducing glucose uptake in pathological conditions. Our data demonstrating TXNIP overexpression and co-localization with aggregates of Aβ and p-tau at the tissue level indicate a likely contributing link between TXNIP function and AD progression.

Most of the existing knowledge about the links between AD and TXNIP imply a causative role of AD pathology in the initiation of TXNIP overexpression. Influenced by multiple stimuli at transcriptional and post-transcriptional levels, TXNIP protein is directly induced by chronic ROS generation in the stressed AD tissue bearing tangles, Aβ plaques, and degenerative alterations [47, 48]. Alternatively, the membrane receptor for glycation end-products (RAGE) has been also suggested to mediate TXNIP induction by Aβ in rat cerebral capillary endothelial cell lines [49]. The significance of this involvement is supported with data from human AD brains presenting RAGE co-localization with TXNIP in brain astrocytes [50]. Whereas our double-staining data do not provide information about the exact link, the observed TXNIP colocalization with p-tau and Aβ plaques cannot rule out a direct TXNIP contribution to Aβ or tau pathology. Based on the earlier experimental data, TXNIP has been found to increase human islet amyloid polypeptide (IAPP) mRNA through miR-124a suppression [51] and enhanced tau phosphorylation in SH-SY5Y cells exposed to okadaic acid [52]. A more recent study also demonstrated that tau phosphorylation at Ser202/Thr205 in $A\beta_{1-42}$ exposed SH-SY5Y cells depends on TXNIP activation [31].

While further clarification is required to demonstrate a direct TXNIP contribution to Aβ or tau pathology, the significance of oxidative stress and NLRP3 inflammasome stimulation in engaging microglial inflammatory responses to escalate AD progress is beyond dispute. TXNIP named for its inhibitory action on TRX, is a strong amplifier of oxidative stress. Additionally, our TRX immunoblotting assay on cortices obtained from aged AD patients showed a remarkable decline in its protein expression, rendering brain tissue more vulnerable to neurodegeneration. This is well corroborated with earlier human data indicating a significant decrease in TRX levels in the amygdala as well as hippocampus/parahippocampal gyrus of AD brains [53], and highlighting TRX in cerebrospinal fluid as an efficient biomarker for AD progress [54].

Our immunoblotting evaluation of NLRP3 inflammasome related molecules ASC, cleaved caspase-1 and cleaved IL-1 β showed a significant elevation in the AD samples. Considering the coincident TXNIP overexpression, this suggests remarkable NLRP3 inflammasome stimulation by TXNIP. To further confirm this link, immunostaining of IL-1β was greater in AD than non-AD samples, and was highly localized in the vicinity of Aβ plaques. This strengthens our hypothesis that there is a close link between TXNIP/IL-1β and the molecular hallmarks of AD. The NLRP3 inflammasome as a strong deteriorating inflammatory effector of TXNIP has been extensively documented to contribute to AD pathology, particularly through IL-β production [55]. Nevertheless, most of the existing evidence has been acquired from murine models of AD.

Human and mouse microglial cells exposed to Aβ release large amounts of IL-1β [56, 57]. Microglial release of IL-1β may increase the processing of the amyloid-β protein

precursor, ultimately leading to further amyloid deposition [58]. The profound IL-1β release in the vicinity of Aβ has been shown to result from NRRP3 inflammasome activation as a consequence of receptor-mediated sensing of insoluble materials [59]. The implication of NLRP3 inflammasome activation in AD has been recently confirmed in animal model, showing significant reductions in AD-related neuropathology by an inflammasome inhibitor [60]. Recent human studies have shown high expression of NLRP1 and NLRP3 in monocytes from AD subjects [61]. A previous study of the NLRP3 inflammasome in human AD brain specimens demonstrated marked caspase-1 and IL-1β overexpression [62], but did not measure NLRP3 inflammasome activation. Our immunoblot probing of the NLRP3 inflammasome did not show any increase in AD brains. Due to the lack of comparative data with human samples this may not contradict with the murine studies [63]; nor does it rule out the NLRP3 inflammasome assembly and activation. In this connection, our findings may conclude the oligomer assembly and cleavage activity has increased enough to cause a significant rise in IL-1β cleavage from its precursor.

In brief, this study presents clinical evidence of the association of AD histological hallmarks with the TXNIP/TRX system and inflammasome activation (Fig. 5). Further investigation is needed to clarify the potential for TXNIP as a multifunctional therapeutic target. Considering the recently-emerged perspective suggesting that aberrant metabolic and electrical activity in particular brain regions in initiate AD pathogenesis [64], TXNIP as a pluripotent molecule with a well-established role in metabolism and insulin function may be involved in more ways than we have examined here. Here, by providing clinical evidence in AD cortex with a close TXNIP/inflammasome/IL-1β association, we focused on inflammatory effectors. Conclusively, our results underline the important role of TXNIP-NLRP3 activation in AD brains. Further investigations are still required to determine the specific contribution and the mechanism of TXNIP associated NLRP3 activation in AD pathogenesis. The involvement of TXNIP in insulin-associated metabolic derangements may provide additional information regarding TXNIP as an upstream instigating signal in AD pathology.

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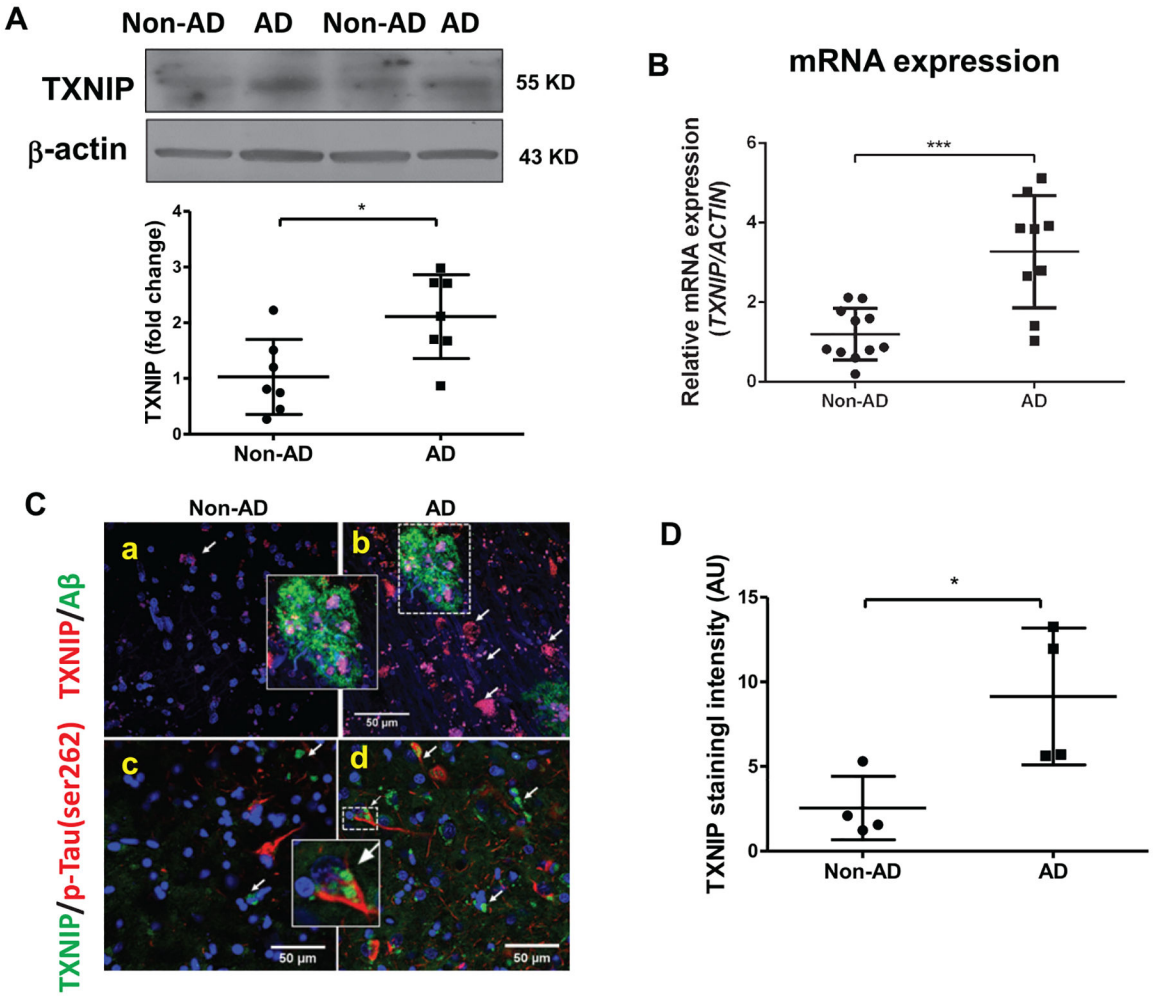

Fig. 1.

TXNIP transcription, expression, and co-localization in cortices of postmortem human AD brains. Frontal and temporal cortical samples were collected from AD and non-AD brains and were subjected to TXNIP analysis. Immunoblotting analysis on frontal specimens revealed a significant (*$p < 0.05$) increase in TXNIP expression in AD brains compared to non-AD counterparts (A). The mRNA levels also significantly (***$p < 0.001$) increased in AD brains, as compared to non-AD brains (B). TXNIP was then examined for co-localization with Aβ or p-tau within the temporal cortices of AD brains as a hallmark of AD pathology (C). Sections were immunostained with anti-Aβ and anti-TXNIP antibodies respectively. Representative images display higher expression and co-localization of Aβ (green) and TXNIP (red) in AD compared to non-AD brain (a-b). Separate sections were also immunostained for *p*-tau and TXNIP demonstrating higher expression and co-localization of TXNIP (green) and *p*-tau ser262 (red) in AD compared to non-AD brains (c-d). Boxed area shows the enlarged view of co-localization of TXNIP with Aβ and *p*-tau. The arrow indicates the immunopositive signals for TXNIP. Scale bar = 50 μm. Labeled intensity and labeled positive area also significantly increased in AD brains, as compared to non-AD brains (D). The arrow indicates the immunopositive signals for TXNIP. Values are expressed as median ± SD for TXNIP signal intensity and mean ± SD for other bars.

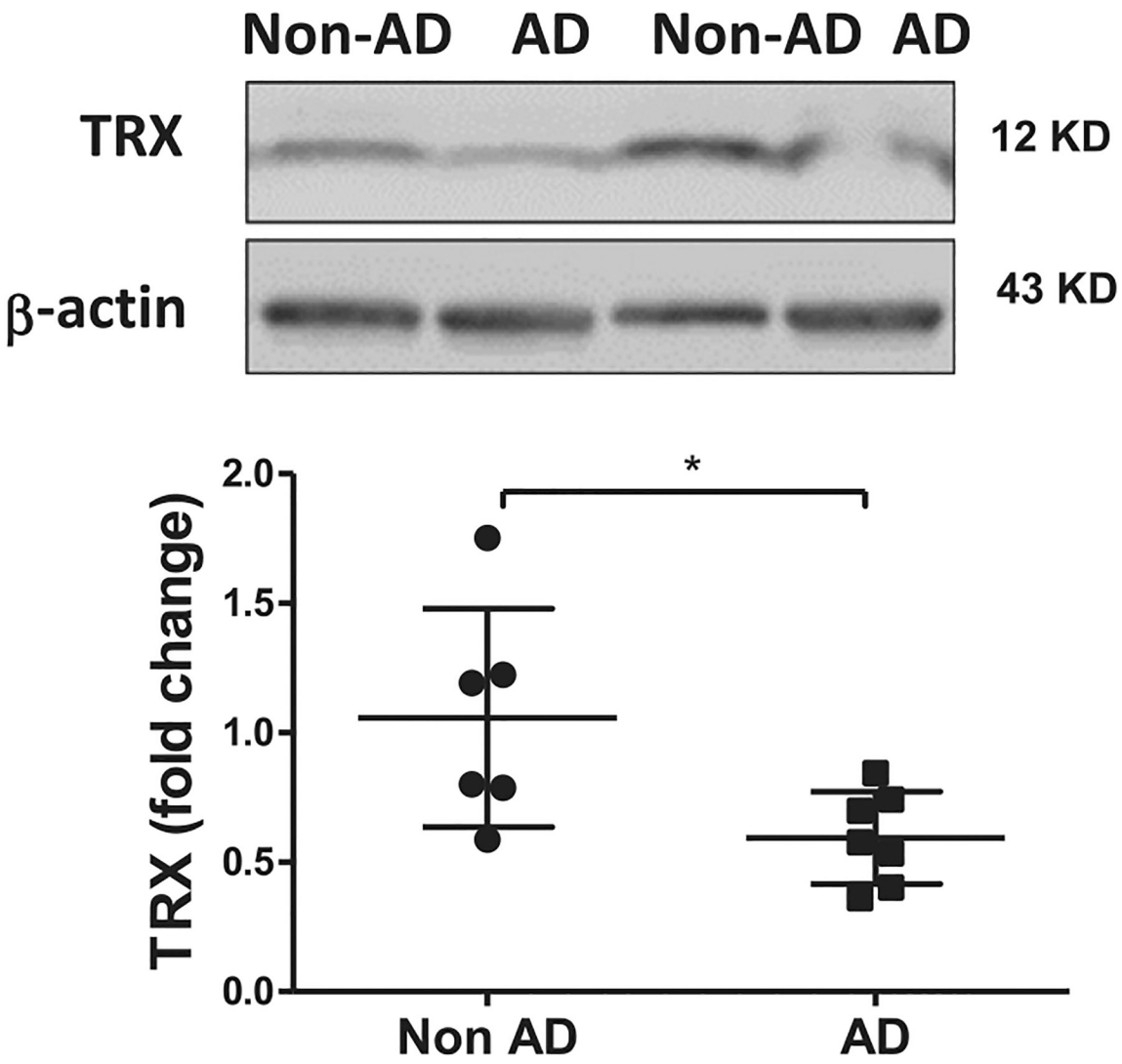

Fig. 2.

TRX expression in cortices of postmortem human AD brains. Frontal cortical samples were collected from AD and non-AD brains were subjected to TRX analysis. Immunoblotting analysis showed a significant reduction in TRX expression in AD brains compared to non-AD (*$p < 0.05$). Values are expressed as mean ± SD.

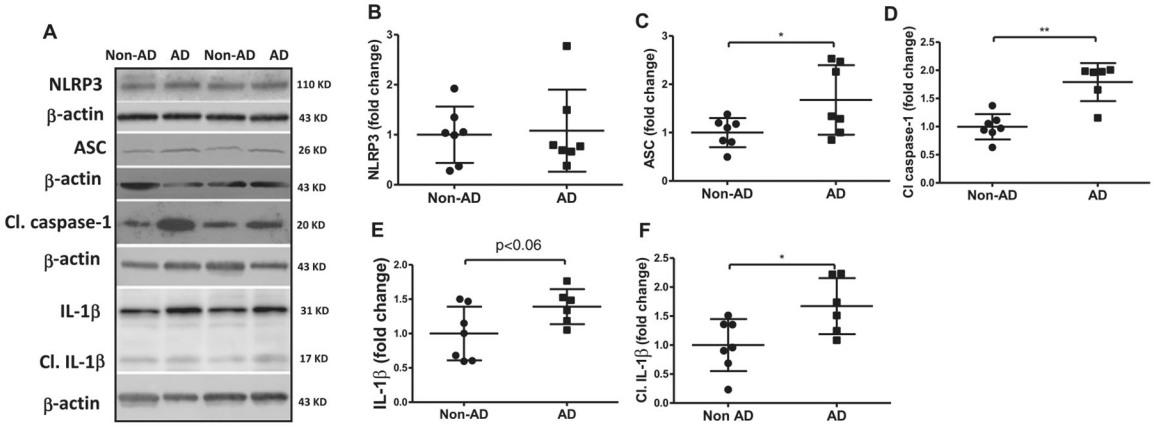

Fig. 3.

Immunoblotting assay of principal constituents of acute NLRP3 inflammasome activation in cortices of postmortem human AD brains. Representative (A) and quantitative analysis of NLRP3 (B), ASC (C), cleaved caspase-1 (D), pro-IL-1β (E), and cleaved IL-1β (F) protein expression demonstrate the corresponding differences between frontal cortices in AD and non-AD brain. Accordingly, AD pathology is associated with enhanced caspase-1 and IL-1β maturation. Values are expressed as mean ± SD. *$p < 0.05$ and **$p < 0.01$ versus non-AD brains.

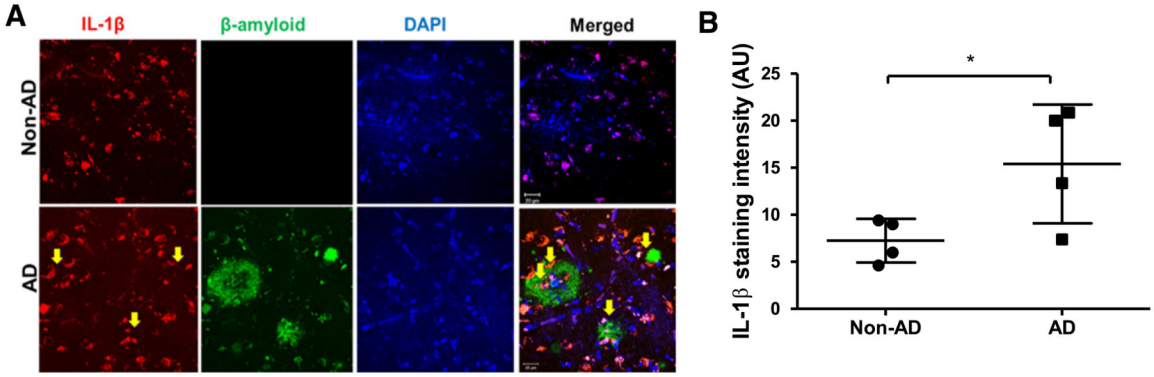

Fig. 4.

Immunostaining of IL-1β in the temporal cortices of postmortem human AD brains. Sections were obtained from temporal cortices of AD and non-AD brains and examined for IL-1β immunostaining and co-expression with Aβ. IL-1β (red) shows higher expression and co-localization with Aβ (green) in AD compared to non-AD control brain (A). IL-1β intensity analysis showed significantly higher IL-1β immunopositive staining in the temporal cortices of postmortem human AD brains (B). The arrow indicates the immunopositive signals for IL-1β, which then is located around Aβ. Values are expressed as mean ± SD.

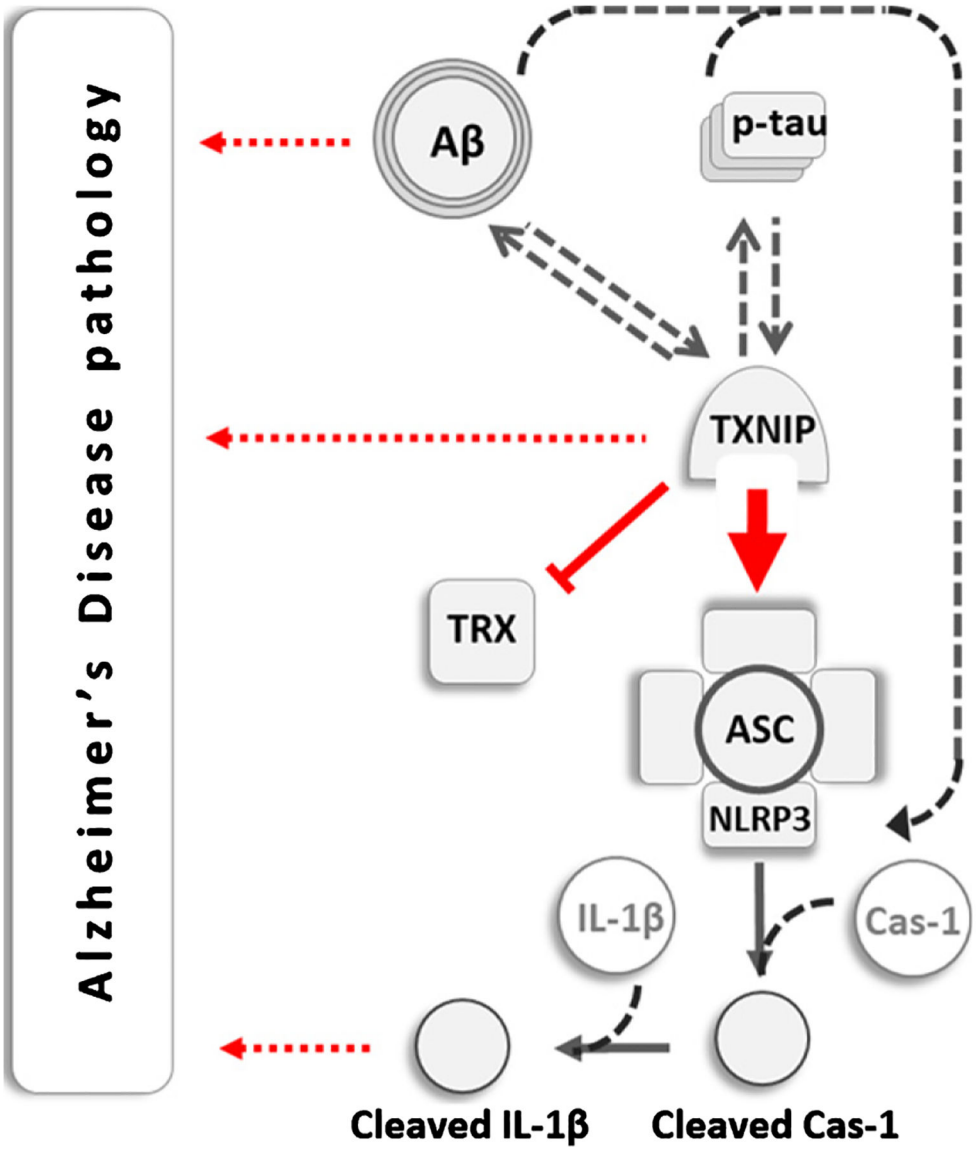

Fig. 5.

Schematic description of presumable link between TXNIP and AD pathology. TXNIP co-localization with Aβ plaques or p-tau in temporal cortices of Alzheimer's disease (AD) patients suggest a causative link between TXNIP and AD pathology. As determined by dotted arrows it is not yet clear whether the pathological aggregates may boost TXNIP expression or TXNIP precedes Aβ or tau buildup. TXNIP activity has an established role to propagate oxidative stress and inflammation. Through direct binding to TRX, TXNIP impedes TRX radical scavenging activity. Recently, TXNIP has been found to interact with NLRP3 inflammasome and improve its cleavage activity to cleave precursors of capase-1 and IL-1β. IL-1β over-expression in the vicinity of Aβ aggregates suggest a prior activation of the NLRP3 inflammasome induced by either TXNIP overexpression or aggregated Aβ itself. TXNIP, thioredoxin interacting protein; TRX, thioredoxin; Aβ, amyloid-β protein;

p-tau, phosphorylated tau protein; ASC, apoptosis-associated speck-like protein; Cas-1, caspase-1; IL-1β, interleukin-1β.

Table 1

Demographic parameters for the postmortem human brain tissue samples

	For Western blotting		
	Age	**Sex**	**PMI**
Non AD (*n* = 6–7)	81 ± 9.18	4 male, 3 female	<4 h
AD (*n* = 6–7)	83 ± 7.19	3 male, 4 female	<4 h
For Real-time PCR			
Non AD (*n* = 11)	76 ± 13.78	8 male, 3 female	<4 h
AD (*n* = 9)	79 ± 10.23	3 male, 6 female	<4 h
For Immunohistochemistry			
Non AD (*n* = 4)	88 ± 2.98	2 male, 2 female	3–8 h
AD (*n* = 4)	83 ± 2.06	2 male, 2 female	3–8 h