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Txnip mediates glucocorticoid-activated NLRP3 inflammatory signaling in mouse microglia

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ABSTRACT

Many studies indicate that chronic stress and excessive stress hormone can cause an inflammatory response. Thioredoxin-interacting protein (Txnip) as an endogenous thioredoxin inhibitor suppresses thioredoxin-produced antioxidant effects. Txnip was also found to interact with nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3), which activates NLRP3 inflammasome and promotes inflammatory processes. Recently our laboratory found that chronic stress can increase Txnip protein levels in mouse brain, indicating that Txnip may mediate chronic stress-induced inflammation. Microglia play an important role in neuroinflammation. The purpose of this study is to investigate the effect of chronic stress hormone treatment on Txnip and NLRP3 inflammasome signaling in cultured microglia cells. Our result showed that chronic treatment with stress hormone corticosterone increased Txnip protein levels and Txnip-NLRP3 binding in N9 mouse microglia, in primary cultured mouse microglia and in mouse brain. Our result also showed that chronic corticosterone treatment increased procaspase-1 cleavage, caspase-1 activity and interleukin-1β release in N9 microglia. Using CRISPR/Cas9 method we found that knocking out Txnip inhibited corticosterone-increased caspase-1 activity and interleukin-1β release. Our results suggest that chronic corticosterone treatment upregulates Txnip and increases Txnip-NLRP3 binding, which activates NLRP3 inflammasome, resulting in activation of caspase-1 and in further releasing of interleukin-1β. It is therefore likely that Txnip-activated NLRP3 inflammasome contributes to corticosterone-caused neuroinflammation.

1. Introduction

Stress is an innate bodily response to environmental threat. The hypothalamic-pituitary-adrenal (HPA) axis regulates the stress response process. Once the HPA axis is activated during stress, corticotropin-releasing hormone is released from the paraventricular nucleus of the hypothalamus, resulting in adrenocorticotropin hormone secretion from the anterior lobe of the pituitary. The release of adrenocorticotropin into systemic circulation leads to secretion from the adrenal cortex of stress hormone glucocorticoids such as corticosterone (CORT) in rodents and cortisol in humans. These glucocorticoids can subsequently target brain and other organs (Mormede et al., 2007). Short-term stress is generally beneficial and helps the body to cope with such threats. However, excessive and prolonged stress may initiate detrimental effects on the brain, which increases the risk of development of various psychiatric disorders (Marin et al., 2011; McEwen, 2004; Tafet and Bernardini, 2003). For example, chronic stress was found to reduce dendritic spine formation, dendritic length and dendritic branching in rodent hippocampus and medial frontal cortex (García-Rojo et al., 2017; Michelsen et al., 2007; Radley et al., 2013). Brain derived neurotrophic factor (BDNF) contributes significantly to neuronal survival and differentiation. It was found that BDNF levels were reduced by chronic stress in rat hippocampus (Di Liberto et al., 2017; Kumar and Mondal, 2016; Luo et al., 2016). Chronic treatment with glucocorticoids was also found to increase oxidative stress and induce apoptosis in primary cultured rat hippocampal neurons, HT22 mouse hippocampal cells and rat pheochromocytoma PC12 cells (Bharti et al., 2018; Lu et al., 2003; Quiros et al., 2008; Tang et al., 2013).

Abbreviations: ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; BDNF, brain derived neurotrophic factor; CORT, corticosterone; DMEM, Dulbecco's Modified Eagle Media; ECL, enhanced chemiluminescence; FBS, fetal bovine serum; HPA, hypothalamic-pituitary-adrenal; HRP, horseradish peroxidase; IL, interleukin; NLRP3, nucleotide-binding oligomerization domain-like receptor protein 3; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulphate; sgRNAs, single guide RNAs; Trx, thioredoxin; TrxR, thioredoxin reductase; Txnip, thioredoxin-interacting protein

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These findings suggest that chronic stress and chronic glucocorticoid treatment can impair brain and cause abnormal neuroplasticity.

Inflammation is defined as an immune response of body tissues to pathogens, damaged cells and other toxins (Chen et al., 2017). Chronic stress can increase expression and release of pro-inflammatory cytokines in brain. It has been found that pro-inflammatory cytokines interleukin (IL)-1β, TNF-α and IL-6 levels were increased in hippocampus and frontal cortex of mice subjected to chronic unpredictable stress for three weeks (Su et al., 2018). Chronic unpredictable stress was also found to increase IL-1β and TNF-α in rat hippocampus and frontal cortex (Sahin et al., 2016). Clinically, stress hormone cortisol is commonly used to inhibit inflammation processes. However, larger amounts of cortisol were found to increase pro-inflammatory cytokine levels in human monocytes (Yeager et al., 2011). In animal studies, chronic treatment with CORT for 21 days was shown to increase mRNA levels of inflammatory cytokines TNFα and IL-13, and cyclooxygenase-2, an enzyme related to inflammation in mouse liver (Ma et al., 2018). Chronic CORT treatment for three days was also found to increase TNFα mRNA levels in cultured rat hippocampal slices (Kurek et al., 2016) and to increase endotoxin lipopolysaccharide-induced TNF-α and IL-1β levels in rat frontal cortex and hippocampus (Munhoz et al., 2010). These findings suggest that both chronic stress and chronic CORT treatment can activate inflammatory processes.

Thioredoxin-interacting protein (Txnip) is an endogenous inhibitor for antioxidant protein thioredoxin and can promote oxidative stress (Spindel et al., 2012). Nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) is critical in mediating inflammatory process. Txnip has been found to interact with NLRP3, which activates NLRP3 inflammasome forming and facilitates inflammatory processes (Schroder et al., 2010). Recently, our laboratory found that mice exposed to chronic unpredictable stress exhibited increased Txnip protein levels in hippocampus and frontal cortex (Zhou et al., 2019), suggesting that Txnip may mediate chronic stress-induced oxidative stress and neuroinflammation. In the nervous system, NLRP3 is mainly expressed in microglia and astrocyte (Mamik and Power, 2017). Microglia play a major role in neuroinflammation. In the present study, we first investigated the effect of chronic CORT treatment on Txnip protein levels and Txnip-NLRP3 binding activity in mouse microglia. Second, we investigated the effect of chronic CORT treatment on NLRP3 inflammasome-mediated signaling, and determined if knocking down Txnip inhibits CORT treatment-induced inflammatory process in mouse microglia.

2. Materials and methods

2.1. Cell culture and drug treatment

N9 mouse microglia cells were generously provided by Professor Maria-Grazia Martinoli (Universite du Quebec a Trois-Rivieres, Quebec, Canada). N9 cells were cultured in Dulbecco's Modified Eagle Media (DMEM) (Life Technologies Inc, Burlington, ON, Canada) supplemented with 10% fetal bovine serum (FBS) (Fisher Scientific, Burlington, Ontario, Canada) and 1% penicillin/streptomycin (Life Technologies Inc, Burlington, Ontario, Canada), and kept in incubator under 5% carbon dioxide at 37 °C.

Primary culture of mouse microglia was performed as described previously (Kauppinen et al., 2011; Zhu et al., 2015). Embryonic fetuses were removed from the uterus of mouse with 17–18 day gestation. The cerebral cortex was dissected, minced finely and transferred to 15 ml tube. The tissue was digested with 500 µl of 0.25% trypsin (Life Technologies Inc, Burlington, Ontario, Canada) for 3 min at 37 °C. Digestion process was terminated with 2 ml of DMEM media containing 10% FBS and 1% streptomycin/penicillin. After the supernatant was removed, tissue precipitation was resuspended with DMEM media containing 10% FBS and 1% streptomycin/penicillin. Media was changed at day 2, 4 and 7. To collect microglia, flasks were vigorously tapped on the bench top and collected in a 15 ml tube. Cells were cultured at a density of 3×10^5 cells on a 6-well plate and maintained at 37 °C under 5% carbon dioxide for all of experiments except one for cell viability assay in which cells were cultured in 96-well plates. Microglia comprise approximately 95% of the total cultured cells as described in our previous publication (Zhu et al., 2015).

Microglia were treated with CORT (MilliporeSigma Canada Co. Oakville, Ontario, Canada) at 0.5, 1, and 5 µM for 5 days. Five day-treatment was intended to mimic chronic treatment with CORT in cultured cells. The concentrations of CORT were used to represent CORT plasma concentration after chronic stress in mice (Lehmann et al., 2013; Lim et al., 2016; Tang et al., 2015; Zu et al., 2017). CORT was dissolved in dimethyl sulfoxide (DMSO). The final DMSO concentration in cultured medium was 0.1% in vehicle and CORT-treated cells. During drug treatment, the medium was changed every other day. Medium always contain either vehicle or CORT.

2.2. Chronic unpredictable stress

Male C57BL/6 mice with 20–28 g at eight-week-old age were purchased from Charles River Canada (Montreal, Canada). The detailed procedure for chronic unpredictable stress has been described in our previous publication and approved by the Animal Care Committee of the University of Manitoba, Canada (Zhou et al., 2019). Briefly, mice in stress group (N = 10) were exposed to two of 9 different stressors including restraint, cold swimming, overnight illumination, foot shock, clamping tail, wet cage, cage tilt, shaking and water deprivation every day for 28 days. Mice in control group (N = 10) were kept in their home cages without any disturbance. After stress procedure was completed, mice were put in CO_2 chamber for 20 s and then decapitated. The brain was isolated, and frontal cortex and hippocampus were dissected and immediately frozen in dry ice and kept in −80 °C freezer.

2.3. Immunoblotting analysis

After washed twice with phosphate buffer saline (PBS), cultured cells were scraped in 500 µl of PBS, and collected in an Eppendorf tube. These cells were centrifuged at 1000 *g* for 5 min at 4 °C and the supernatant was removed. The pellet was resuspended with 50 µl of ice cold lysis buffer including 20 mM HEPES (pH 7.5), 250 mM NaCl, 30 mM $MgCl_2$, 0.1 mM EGTA, 0.5 mM EDTA, 20% glycerol, 1% Nonidet P40 and 1 × protease inhibitor cocktail (Thermo Scientific, Marietta, OH, USA), vortexed and kept on ice for 1 h. Mouse hippocampus and frontal cortex were homogenized in 10:1 (ml/g) ice-cold lysis buffer and also kept on ice for 1 h. Lysed cells or tissue homogenates were then centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatants were collected and used as protein extract. Protein concentration was determined by Bradford protein assay (Bradford, 1976).

Protein samples were added with a loading buffer [100 mM Tris-HCl (pH 6.8), 200 mM dithiothreitol, 4% sodium dodecyl sulphate (SDS), 0.2% bromophenol blue and 20% glycerol], then loaded to 12% SDS polyacrylamide gel, and subjected to electrophoresis at 120 V for 1 h. Then the proteins were transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA) for 2 h at 220 mA on ice. Membranes were incubated with 5% milk in Tris-buffered saline [10 mM Tris-HCl (pH 7.4) and 0.1% Tween-20] at 22 °C for 1 h, and further incubated with a primary antibody overnight at 4 °C and then with horseradish peroxidase (HRP) secondary antibody at 22 °C for 1 h. The enhanced chemiluminescence (ECL) reagents were used to detect protein bands (Bio-Rad, Dreieich, Germany). The band imaging was captured by the ChemiDoc MP System (Bio-Rad), and analyzed by using Image Lab software (Bio-Rad).

2.4. Co-immunoprecipitation

First protein was pulled down by NLRP3 antibody immobilized to

Protein A/G beads. 100 µg of proteins were incubated with mouse monoclonal NLRP3 antibody (Adipogen, CA, USA) overnight at 4 °C. Then 7 µl of protein A/G beads (Santa Cruz Biotechnology, USA) were added to protein-antibody complex and incubated for 2 h at 4 °C. Protein-bead mixture was then centrifuged at 1000 g for 30 s and supernatants were removed. The beads were washed three times with cold buffer containing 50 mM Tris-HCl (pH = 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100 and protease inhibitor cocktail (Thermo Scientific). After final wash, 15 µl of buffer containing 50 mM Tris–HCl (pH 6.8), 2% SDS, 0.1% bromophenol blue and 10% glycerol was added to the beads. Secondly, pulled-down protein was run in SDS polyacrylamide gel, transferred to membranes and analyzed by western blot with Txnip antibody. The samples were heated at 95 °C for 5 min and were subjected to SDS polyacrylamide gel and transferred to polyvinylidene fluoride membranes. The membrane was incubated with rabbit polyclonal Txnip antibody (Abcam Inc., Toronto, ON, Canada) at 4 °C overnight followed by HRP secondary antibody at 22 °C for 1 h. The ECL reagents were used to detect protein bands (Bio-Rad, Dreieich, Germany). The band imaging was captured and analyzed by the ChemiDoc MP System and Image Lab software (Bio-Rad). Then membrane was stripped and re-probed with an antibody for NLRP3 for checking loading control.

2.5. Spectrophotometric assay for measuring caspase-1 activity

The caspase-1 activity was measured as described previously (Lopez-Castejon et al., 2013). 100 µg of N9 cell extracts were added to 50 µl of 2X reaction buffer containing 50 mM Hepes (pH = 7.4), 100 mM NaCl, 10 mM dithiothreitol, 1 mM EDTA and 10% v/v glycerol. Then 50 µM of fluorogenic caspase-1 substrate Z-YVAD-AFC (Cayman Chemical, CA, USA) was added to this reaction mixture and incubated for 2 h at 37 °C. After incubation, the fluorescence was measured at 400 nm (excitation wavelength) and 505 nm (emission wavelength).

2.6. Enzyme-linked immunosorbent assay

IL-1β protein levels in cultured medium were analyzed with an enzyme-linked immunosorbent assay kit according to manufacturer's instructions (R&D systems, Minneapolis, MN, USA). Briefly, the 96-well plate was coated with capture antibody at 4 µg/ml concentration and incubated overnight at 4 °C. Then the antibody was removed and plate was washed 3 times with washing buffer containing 0.05% Tween-20 in PBS. The plate wells were blocked by using blocking buffer containing 1% bovine serum albumin in PBS for 1 h at room temperature. The plate was washed 3 times, and added with 100 µl of sample and incubated for 2 h at room temperature. After washing, 100 µl of detection antibody at a final concentration of 500 ng/ml was added and incubated for 2 h at 22 °C. Then 100 µl of Streptavidin-HRP was added and further incubated for additional 20 min at 22 °C in dark. Plate was washed and 100 µl of substrate solution containing 1:1 mixture of tetramethylbenzidine and H_2O_2 was added to the plate. The plate was incubated in dark for 20 min, and added with 50 µl of stop solution containing 2N H_2SO_4. The optical density was measured by subtracting the readings at 540 nm from readings at 450 nm.

2.7. Measurement of cell viability

Cell viability was determined by measuring the cleavage of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) using MTT Cell Viability Assay Kit (Biotium, Inc. Fremont, California). N9 cells were incubated with 10 µl of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) at 37 °C for 4 h, and then added with 200 µl DMSO to dissolve formazan product. The absorbance was recorded at a wavelength of 570 nm.

2.8. Knocking out Txnip in N9 cells

Txnip knocking out was performed using CRISPR-Cas9 method as described in our previous publication (Bharti et al., 2018). Lentivector containing Txnip single guide RNAs (sgRNAs) and CRISPR/Cas9 was generously provided by Dr. Eftekhar Eftekharpour (University of Manitoba, Winnipeg, Canada). Lentivector containing scrambled sgRNAs and CRISPR/Cas9 was used as control. Lentivector was packaged with lentivirus performed by Lentiviral Core Service at University of Manitoba. N9 cells were plated in 6-well plates at approximately 50% confluence. Lentiviral particles were added to cultured cells in DMEM medium with 10% FBS and incubated at 37 °C for 24 h. Then medium containing virus particles was discarded and cells were cultured in DMEM medium with 4 µg/ml puromycin. Single clones of stably transfected cells were further cultured for experiments.

2.9. Data analysis

Results were displayed as means ± standard error of the mean. IBM SPSS 24.0 software (IBM, Armonk, New York, USA) was used for data analysis. Differences among means were assessed by one-way analysis of variance with Tukey's honestly significant difference post hoc comparison test. Student's t-test was used for statistical analysis of two groups. Significant differences were determined by p value of less than 0.05.

3. Results

First, the effect of chronic treatment with CORT on Txnip protein levels was analyzed in N9 mouse microglia cells. Our finding showed that chronic treatment with CORT at concentrations of 0.5–2 µM significantly increased Txnip protein levels (Fig. 1A). We further verified that chronic treatment with 1 µM CORT also increased protein levels of Txnip in primary cultured mouse microglia (Fig. 1B).

Secondly, because Txnip can bind to NLRP3 in microglia, we investigated the effect of CORT on Txnip-NLRP3 binding activity using co-immunoprecipitation in N9 cells. NLRP3-binding proteins from protein extract were immuneprecipitated with NLRP3 antibody. Txnip levels in pulled down proteins were measured using immunoblotting analysis with Txnip antibody. The membrane blot was then re-probed with NLRP3 antibody. As shown in Fig. 2A, chronic treatment with

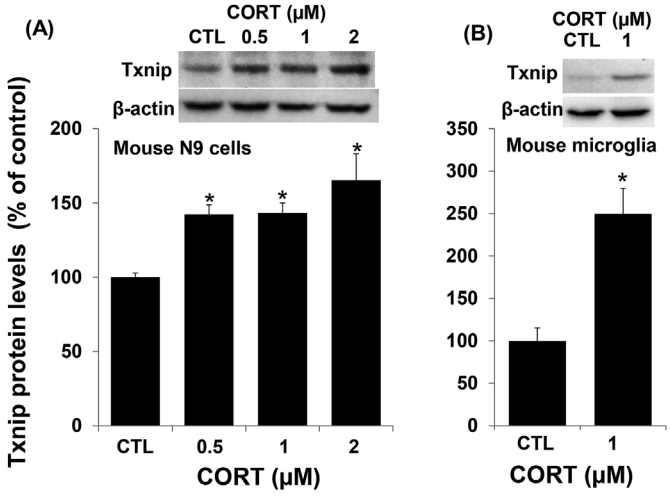

Fig. 1. Chronic corticosterone treatment increases Txnip protein levels in N9 mouse microglia cells and primary cultured microglia cells. N9 microglia (A) (N = 6) and primary cultured microglia (B) (N = 4) were treated with vehicle (CTL) or corticosterone (CORT) for 5 days. β-actin was used as loading control. *p < 0.05 determined by one-way ANOVA followed by Tukey's post-hoc analysis when compared to controls.

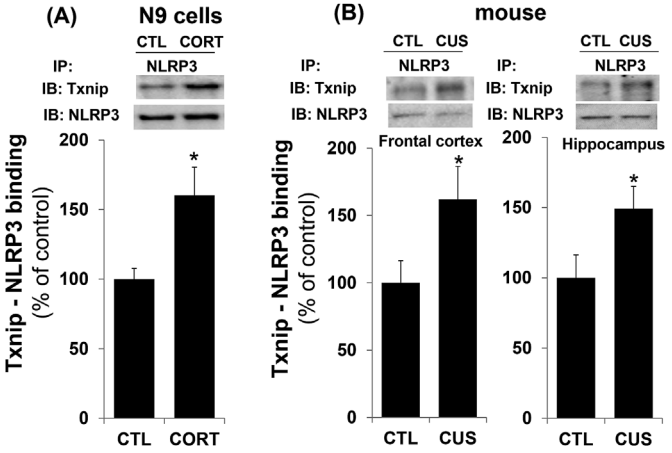

Fig. 2. NLRP3/Txnip binding in corticosterone-treated N9 mouse microglia cells and in hippocampus and frontal cortex of mice exposed to chronic stress. (A) N9 microglia were incubated with CORT at 1 μM for 5 days (N = 5). (B) Mice were exposed to chronic unpredictable stress (CUS) for 28 days (N = 10). Protein was extracted from N9 cells or mouse frontal cortex and hippocampus. NLRP3 was immunoprecipitated (IP) by NLRP3 antibody, followed by immunoblotting analysis (IB) using Txnip antibody. Then membrane was stripped and re-probed with an antibody for NLRP3 for checking loading control. *p < 0.05 determined by Student's t-test when compared to controls.

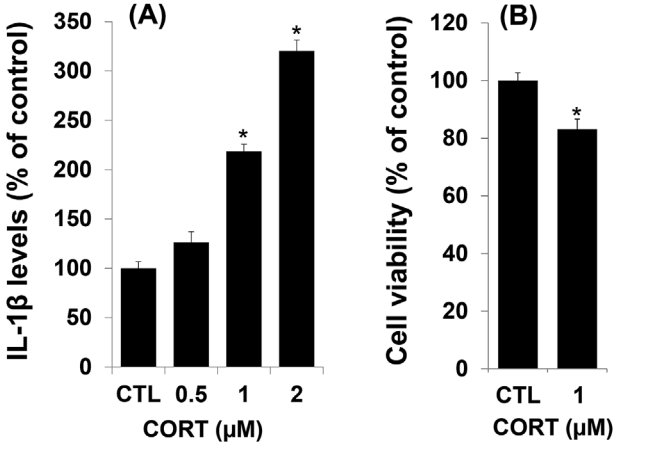

Fig. 4. Chronic corticosterone treatment increases IL-1β protein levels and decreases cell viability in N9 cells. (A) N9 microglia were incubated with CORT at 0.5, 1 and 2 μM for 5 days. Protein levels of IL-1β in cultured medium were measured using an enzyme-linked immunosorbent assay (N = 4). *p < 0.05 determined by one-way ANOVA followed by Tukey's post-hoc analysis when compared to controls. (B) N9 cells were incubated with CORT at 1 μM for 5 days. Cell viability was determined using MTT method (N = 6). *p < 0.05 determined by Student's t-test.

CORT at 1 μM for 5 days significantly increased Txnip-NLRP3 binding in N9 cells. Previously we found that chronic unpredictable stress increased Txnip protein levels in mouse frontal cortex and hippocampus (Zhou et al., 2019). In the present study, we also verified the effect of chronic stress on Txnip-NLRP3 binding in mouse frontal cortex and hippocampus. We found that mice subjected to chronic unpredictable stress for 28 days exhibited increased Txnip-NLRP3 binding in both frontal cortex and hippocampus when compared to controls (Fig. 2B).

Thirdly, because NLRP3 inflammasome can cleave procaspase-1 to caspase-1, activate caspase-1activity and increase IL-1β release, we investigated the effect of CORT on procaspase-1 cleavage, caspase-1activity and IL-1β release in N9 cells. Our finding showed that chronic treatment with CORT at 0.5–2 μM for 5 days significantly reduced procaspase-1 protein levels (Fig. 3A), suggesting that CORT treatment increased procaspase-1 cleavage. Our finding also showed that chronic treatment with 0.5 μM CORT had no effect on caspase-1 activity, but 1 and 2 μM CORT significantly increased caspase-1 activity in N9 cells (Fig. 3B). Chronic treatment with 1 and 2 μM CORT also significantly elevated IL-1β levels in cultured medium (Fig. 4A). We further found that chronic treatment with CORT at 1 μM reduced cell viability (Fig. 4B). These studies suggest that CORT treatment can activate caspase-1 and release IL-1β in N9 cells.

Finally, to investigate the role of Txnip in chronic CORT treatment-induced caspase-1 enzyme activation and IL-1β release, we investigated the effect of Txnip knockout on CORT-increased caspase-1 activation and IL-1β release in N9 microglia. We found that Txnip protein levels were much lower in N9 microglia transfected with Txnip sgRNAs/CRISPR Cas9 than in microglia transfected with scrambled sgRNA/CRISPR Cas9 (control group) or in wild-type cells (Fig. 5). This result suggests that Txnip sgRNAs/CRISPR Cas9 can knock out Txnip expression. Our finding also showed that although chronic treatment with 1 μM CORT increased caspase-1 activity in control group, this treatment did not produce any effect on caspase-1 activity in Txnip knockout cells

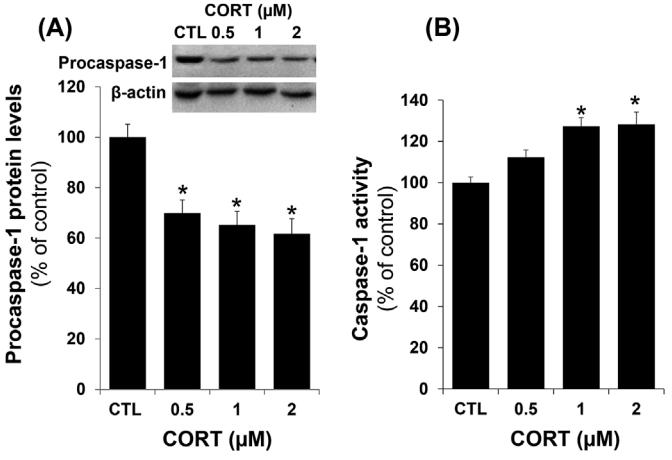

Fig. 3. Chronic corticosterone treatment decreases procaspase-1 protein levels and increases caspase-1 activity in N9 mouse microglia cells. N9 microglia were incubated with CORT for 5 days. (A) Protein levels of procaspase-1 were measured with procaspase-1 antibody (N = 7). β-actin was used as loading control. (B) Activity of caspase-1 was measured using spectrophotometric assay (N = 8). *p < 0.05 determined by one-way ANOVA followed by Tukey's post-hoc analysis when compared to controls.

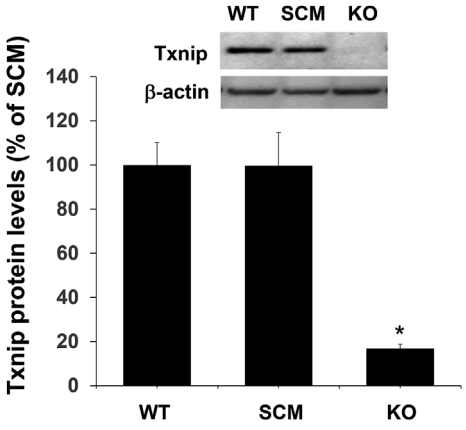

Fig. 5. Knocking out Txnip gene in N9 microglia. N9 microglia were transfected with lentivectors with Txnip sgRNAs/CRISPR/Cas9 (KO). Lentivectors with scrambled sgRNAs/CRISPR/Cas9 (SCM) were used as control (N = 3). Wild-type (WT) N9 cells were used as control. β-actin was used as loading control. *p < 0.05 determined by one-way ANO VA followed by Tukey's post-hoc analysis when compared to WT and SCM groups.

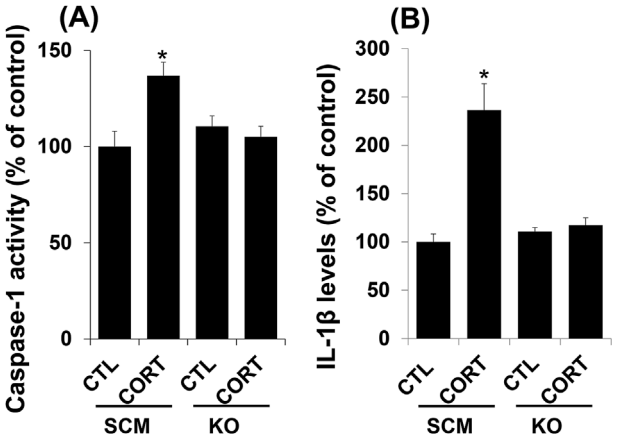

Fig. 6. Effect of Txnip sgRNAs on CORT-increased caspase-1 activity and IL-1β release in N9 microglia. N9 microglia were transfected with Txnip sgRNAs (KO) or scrambled sgRNAs (SCM) and further incubated with CORT at 1 μM for 5 days. (A) Activity of caspase-1 was measured using spectrophotometric assay (N = 6). Protein levels of IL-1β in cultured medium were measured using an enzyme-linked immunosorbent assay (N = 4). *p < 0.05 determined by one-way ANOVA followed by Tukey's post-hoc analysis when compared to controls.

(Fig. 6A), suggesting that Txnip knockout inhibited CORT-increased caspase-1 activity. Our finding also showed that Txnip knockout inhibited 1 μM CORT-increased IL-1β levels in culture medium (Fig. 6B). Our finding suggests that Txnip may mediate CORT-activated caspase-1 activity and CORT-increased IL-1β release.

4. Discussion

In the present study, our results showed that chronic CORT treatment increased protein levels of Txnip in both N9 mouse microglia cells and primary cultured mouse microglia. Previously our laboratory had showed that protein levels of Txnip were increased by chronic treatment with CORT in cultured mouse neuronal cells (Bharti et al., 2018). These findings taken together suggest that CORT upregulates Txnip not only in neurons, but also in microglia.

Many studies using co-immunoprecipitation have shown that Txnip can directly bind to NLRP3 (Tseng et al., 2016; Zhou et al., 2010), suggesting that Txnip may further interact with NLRP3. NLRP3 is a component of NLRP3 inflammasome which is a protein complex including NLRP3, apoptosis-associated speck like protein containing a caspase recruitment domain (ASC) and procaspase-1 (Lamkanfi and Kanneganti, 2010). It has been found that binding of Txnip to NLRP3 can further induce NLRP3 inflammasome forming. Treatment with homocysteine not only increases Txnip and NLRP3 co-localization but also increases NLRP3 and ASC co-localization, while knocking down Txnip with Txnip siRNA inhibits NLRP3 and ASC co-localization in cultured podocytes (Abais et al., 2014), suggesting that Txnip-NLRP interaction may facilitate NLRP3 inflammasome assembly. In the nervous system, NLRP3 is mainly expressed in glia (Mamik andPower, 2017). In the present study, our data showed that chronic CORT treatment increased Txnip-NLRP3 binding in N9 microglia cells. Because CORT concentration was increased in serum of mice exposed to chronic unpredictable stress (Tang et al., 2015; Zu et al., 2017), we further analyzed Txnip-NLRP3 binding in brain of mice exposed to chronic unpredictable stress. We found that Txnip-NLRP3 binding was increased in hippocampus and frontal cortex of mice subjected to chronic unpredictable stress when compared to controls. NLRP3-ASC binding activity was also found to be increased by chronic unpredictable stress in rat hippocampus (Yue et al., 2017). These findings indicate that chronic stress-increased CORT may further increase Txnip levels, subsequently promoting Txnip-NLRP3 binding and facilitating NLRP3 inflammasome assembly.

NLRP3 inflammasome activation can cleave procaspase-1 and activate caspase-1, converting pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18, which accelerate proinflammatory responses (Lenart et al., 2016). In the present study, our data showed that chronic CORT treatment reduced procaspase-1 levels and increased caspase-1 enzyme activity. This finding suggests that CORT may induce cleavage of procaspase-1 and release of mature caspase-1, subsequently inducing activation of caspase-1. Our data further showed that chronic CORT treatment increased IL-1β levels in culture medium, suggesting that CORT may increase IL-1β secretion. We also found that chronic CORT treatment reduced cell viability. Our findings suggest that CORT-upregulated Txnip levels may further activate forming of NLRP3 inflammasome, resulting in caspase-1 activation, IL-1β secretion and cell viability reduction. Previously it was reported that chronic unpredictable stress induced cleavage of procaspase-1, and increased protein levels of IL-1β and IL-18 in mouse brain (Cao et al., 2017; Wang et al., 2018). Chronic restraint stress was also found to increase the protein levels of p20 subunit, a cleaved product of procaspase-1 and protein levels of IL-1β in CA1 region of mouse hippocampus (Song et al., 2018). It has also been found that chronic restraint stress increased mRNA and protein levels of IL-1β and IL-18 in rat prefrontal cortex and hippocampus (Sahin et al., 2016). These findings together suggest that chronic stress increases glucocorticoids, resulting in upregulation of Txnip and activation of NLRP3 inflammasome, subsequently activating caspase-1 and releasing IL-1β and IL-18 in rodents.

To elucidate the role of Txnip in CORT-induced NLRP3 inflammasome activation, we analyzed the effect of Txnip knockout on CORT-increased caspase-1 activity and IL-1β secretion. Txnip gene was knocked out by Txnip sgRNA/Cas9 in N9 microglia cells. Our finding showed that although chronic CORT treatment increased caspase-1 activity and medium IL-1β levels in scrambled sequence transfected N9 microglia, this treatment has no effect on caspase-1 activity and medium IL-1β levels in Txnip sgRNA transfected N9 cells. Our results indicate that Txnip mediates CORT-induced caspase-1 activation and IL-1β release. These findings together indicate that CORT-increased Txnip binds to NLRP3 and facilitates forming of NLRP3 inflammasome, resulting in procaspase-1 cleavage that induces caspase-1 activation and IL-1β releasing. These findings also suggest that Txnip mediates chronic CORT-promoted inflammatory processes.

In conclusion, our results showed that chronic CORT treatment increased Txnip protein levels in cultured mouse microglia cells. Chronic CORT treatment also increased Txnip-NLRP3 interaction in cultured microglia cells. Txnip-NLRP3 interaction was also increased in frontal cortex and hippocampus of mice exposed to chronic unpredictable stress. Further, we found that chronic CORT treatment increased procaspase-1 cleavage, activated capase-1 enzyme activity and increased IL-1β release, and that knocking out Txnip inhibited CORT-induced capase-1 activation and IL-1β release. Our findings indicate that chronic CORT treatment may upregulate Txnip and increase Txnip-NLRP3 binding, facilitating NLRP3 inflammasome forming that promotes procaspase-1 cleavage, activates caspase-1 and releases IL-1β. Our findings also indicate that Txnip-activated NLRP3 inflammasome signaling may mediate the CORT-induced inflammatory process.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://

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