Tripartite motif protein 32 overexpression promotes inflammation and pyroptosis in INS-1 cells via the NF- κ B/NLRP3 signaling pathway under high glucose conditions

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Research Article

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is an inflammatory disease, and the primary features are insulin resistance and β -cell dysfunction. Inflammation and pyroptosis contribute to insulin resistance and β -cell dysfunction. Therefore, it is important to identify novel biomarkers to alleviate inflammation and pyroptosis in T2DM. Tripartite motif protein 32 (TRIM32) belongs to the TRIM family and is involved in inflammation, oxidative stress, apoptosis, and autophagy. Moreover, TRIM32 participates in insulin resistance in skeletal muscle and liver; however, its role in β -cell dysfunction remains elusive. Herein, this study investigated the role of TRIM32 in inflammation and pyroptosis in INS-1 cells.

Methods and Results: This study confirmed for the first time that serum TRIM32 was upregulated in T2DM patients by ELISA, and high glucose-induced TRIM32 expression in INS-1 cells was validated by western blot, quantitative real-time reverse transcriptase-polymerase chain reaction, and immunofluorescence staining. Overexpression of TRIM32 promoted inflammation and pyroptosis in INS-1 cells under high glucose conditions, whereas silencing TRIM32 had the opposite effects. TUNEL staining and lactate dehydrogenase release assays were performed. In addition, BAY 11-7082 blocked the NF- κ B/NLRP3 signaling pathway and significantly attenuated INS-1 cells inflammation and pyroptosis in a high glucose environment.

Conclusions: Overexpression of TRIM32 promoted inflammation and pyroptosis in INS-1 cells partly through the NF- κ B/NLRP3 signaling pathway under high glucose conditions.

1. Introduction

Type 2 diabetes mellitus (T2DM) has emerged as a serious public health issue, and its prevalence has increased at an alarming rate worldwide in recent decades. The International Diabetes Federation (IDF) estimates that in 2021, there were 536 million (aged 20–79 years) people with diabetes worldwide, and by 2045, this figure is expected to reach 783 million [1]. T2DM is a form of complex metabolic disruption, and pancreatic β -cell dysfunction and insulin resistance are central to pathogenesis [2]. T2DM is an inflammatory disease [3], immune cells such as macrophages infiltrate in the islets of T2DM patients, IL-1 β and other proinflammatory cytokines are elevated, the islets develop chronic inflammation, and islet inflammation exerts an important role in pancreatic β -cell dysfunction [4, 5]. High glucose-induced the production and release of IL-1 β were increased in human islets, followed by NF- κ B activation and impaired β cell function [4]. Moreover, activated NF- κ B initiates pro-IL-1 β transcription. Our previous studies confirmed that autophagy reduced oxidative stress-induced NLRP3 inflammasome activation to protect pancreatic islet β -cells from inflammatory damage. The NLRP3 inflammasome is a multiprotein complex and consists of NLRP3, an apoptosis-associated speck-like protein (ASC) and pro-caspase-1. Pyroptosis is an inflammasome-mediated programmed cell death [6]. The NLRP3 inflammasome activates caspase-1, and cleaved caspase-1 cleaves pro-IL-1 β and pro-IL-18 into mature IL-1 β and IL-18 and gasdermin D (GSDMD) into GSDMD-N, which has the membrane pore-forming activity; thus, pyroptosis is characterized by caspase-1 mediated lytic cell death and mature IL-1 β and IL-18 release [7,

8]. NF- κ B regulates NLRP3 expression which is one of two key checkpoints of NLRP3 inflammasome activation [9]. Therefore, NF- κ B, NLRP3 inflammasome, and IL-1 β play an important role in the inflammation and pyroptosis of T2DM.

The richest subfamily of the interesting new gene (RING) E3 ubiquitin ligases, tripartite motif-containing (TRIM) family proteins, plays a significant role in the development of cancer, autoimmune diseases, and inflammatory diseases by regulating NF- κ B transcriptional activity [10, 11]. NF- κ B activation is essential for the transcriptional induction of TRIM37 during hepatitis B virus infection [12], and TRIM8 has been implicated in IL-1 β -induced chondrocyte inflammation in osteoarthritis and inflammation in ischemia-reperfusion brain injury based on its function in regulating NF- κ B activity [13, 14]. The TRIM family member pyrin/TRIM20, as the canonical inflammasome, is implicated in IL-1 β and IL-18 production, and pyroptosis [15, 16]. TRIM32 is involved in cisplatin resistance via NF- κ B signaling regulation in human nonsmall-cell lung cancer [17] and activates NF- κ B signaling and NLRP3 in rheumatoid arthritis [18]. Another study indicated that TRIM32 may promote NF- κ B signaling activation [19]. Furthermore, TRIM32 contributes to insulin resistance in skeletal muscle and liver by inhibiting the PI3K/Akt pathway [20]. A new study showed that TRIM32 expression increases in the glomeruli of diabetic mice, and TRIM32 regulates high glucose-induced apoptosis, oxidative stress, and inflammatory injury in podocytes [21]. The above findings led us to postulate that TRIM32 participates in β -cell inflammation and pyroptosis via the NF- κ B/NLRP3 signaling pathway.

In this study, the results demonstrated that serum TRIM32 was upregulated in T2DM patients and that HG increased INS-1-cell TRIM32 expression. Immunofluorescence staining showed that TRIM32 was localized in the nucleus. TRIM32 overexpression aggravated HG-induced inflammation and pyroptosis in INS-1 cells. Silencing TRIM32 exerted the opposite effects. The NF- κ B/NLRP3 pathway blockade significantly attenuated inflammation and pyroptosis induced by HG in INS-1 cells. Our study provides new insight into the role of TRIM32 in INS-1-cell inflammation and pyroptosis and evidence to indicate its potential value as a therapeutic target for T2DM in the future.

2. Materials And Methods

2.1. Serum specimens

All participants were recruited during clinic visits at the First Affiliated Hospital of Harbin Medical University and Hulan Red Cross Hospital from November 2020 to May 2021. The diagnostic criteria of enrolled T2DM or prediabetes patients were based on the 2021 American Diabetes Association (ADA) criteria. Serum specimens were collected from 81 participants and stored at -80°C for subsequent assays. Participants in each group were matched for mass index (BMI).

2.2. Cell culture

The rat insulinoma cell line (INS-1) used in this study was provided by the Department of Endocrinology, The Second Affiliated Hospital of Harbin Medical University (Heilongjiang, China). INS-1 cells were

cultured in RPMI1640 medium (Invitrogen, USA). RPMI 1640 medium containing 11.1 mM glucose was used as a standard culture condition for INS-1 cells as reported in the literature. Therefore, 11.1 mmol/L glucose served as the control group in this study [22]. The culture medium was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were cultured in a cell culture incubator (humidified at 37°C, 5% CO₂).

2.3. Reagents and antibodies

BAY 11-7082, bicinchoninic acid (BCA) kit, and penicillin/streptomycin were purchased from Beyotime (Shanghai, China). TRIzol reagent and DAPI were obtained from Sigma–Aldrich (USA). The cDNA First-Strand Synthesis Kit and SYBR Green Master Mix were purchased from Roche (Switzerland). LipoFiter 3.0 was obtained from Hanbio Biotechnology (Shanghai, China), and FBS was obtained from Sijiqing Biological Engineering Materials Co., Ltd (Hangzhou, China). Antibodies against TRIM32 were purchased from Bioss (China), NLRP3, NF-κB p65, phospho-NF-κB p65, cleaved caspase-1, ASC, IL-18, and IL-1β were purchased from Wanleibio (Shenyang, Liaoning, China), GSDMD-N and horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibodies were purchased from ABclonal (Wuhan, China).

2.4. Enzyme-linked immunosorbent assay (ELISA)

After the indicated treatment, the concentrations of TRIM32 in the serum samples were examined with commercial ELISA kits according to the manufacturer's instructions. TRIM32 ELISA kits were purchased from Jianglai Biotechnology (Shanghai, China). A microplate reader (Tecan, Switzerland) was used to determine the absorbance (OD value) at 450 nm. A standard curve was drawn, and the TRIM32 or insulin concentration was calculated via the linear equation.

2.5. Total RNA isolation and quantitative real-time PCR (RT-qPCR)

Total RNA was isolated from the cell samples using TRIzol reagent. A First-Strand cDNA Synthesis Kit was used to synthesize the cDNA according to the manufacturer's instructions. qPCR was performed using SYBR Green Master Mix on a CFX96 Real-Time System. The primer sequences used were as follows: Rat TRIM32 F: 5'-CCGGGAAGTGCT AGAATGTC-3' and R: 5'-CAGCGGACACCATTGATGCT-3'; Rat GAPDH F: 5'-ATGCCGCCTGGAGAAACC-3' and R: 5'-GCATCAAAGGTGGAAGAATGG-3'. The mRNA of GAPDH was used as a quantitative control, and relative gene expression was calculated as comparative Ct ($2^{-\Delta\Delta Ct}$).

2.6. Western blot analysis

RIPA lysis buffer containing 1% PMSF was used to extract proteins from cells. Protein concentrations were quantified by a BCA kit. Equal quantities of protein were separated by gel electrophoresis and then transferred to a polyvinylidene difluoride membrane. Sodium dodecyl sulfate (SDS)-polyacrylamide gels (10% and 12%) were used. Membranes were blocked in 5% skim milk powder for 2 h and then incubated with primary antibodies overnight at 4°C. Subsequently, HRP-conjugated secondary antibodies were added at room temperature for 1 h. Protein bands were imaged using an enhanced chemiluminescence

(ECL) detection system (Pierce) according to the supplier's instructions. Primary antibodies against the following proteins were used: TRIM32, NLRP3, NF- κ B p65, phospho-NF- κ B p65, GSDMD, cleaved caspase-1, ASC, IL-18, IL-1 β , and β -actin. Details information on the antibodies is listed in Table 1 (Online Resource 1).

2.7. Immunofluorescence staining

After the indicated treatment, cells were fixed with 4% paraformaldehyde for 30 min, washed with PBS, blocked with 5% bovine serum albumin (BSA) for 30 min, washed again, and then cultured with primary antibody against TRIM32 (1:500) overnight at 4°C in a humidified chamber. Subsequently, the fluorescent secondary antibody was added and incubated for 2 h, and nuclei were counterstained with DAPI for 5 min. The cells were protected from light at room temperature. Finally, the cells were sealed with an anti-fluorescence quencher. Images were taken with a Zeiss LSM 510 META confocal microscope.

2.8. Plasmid construction and cell transfection

For overexpression of TRIM32 in INS-1 cells, full-length TRIM32 was inserted into the pCDNA3.1-CMV-EF1-ZsGreen-T2A-puro vector. Short hairpin RNA (shRNA) sequences targeting mouse Trim32 were inserted into the pCDNA3.1-U6-MCS-CMV-ZsGreen-T2A-puro vector. The shRNA sequences were designed based on the literature [23] and were as follows: 5' to 3': GATCTTCAGGCAAGGTATA. Cell transfection was performed according to the manufacturer's protocol (Hanbio Biotechnology, Shanghai, China). Then, the transfection efficiency was assessed. Images were obtained via photography with a Leica DM 4000B fluorescence microscope.

2.9. Lactate dehydrogenase (LDH) assay

LDH activity in the cell supernatant was determined using an LDH cytotoxicity assay kit (Elabscience, Wuhan, China) following the manufacturer's protocol. It was used to quantify the extent of cell death. Briefly, cell supernatant (50 μ L) and reaction solution (50 μ L) were added to the kit reagent and cultured for 10 min at 37°C, and then a stopping solution was added. The absorbance was measured using a microplate reader (Tecan, Switzerland) at wavelengths of 450 nm and 600 nm.

2.10. TUNEL assay

The cell death rate was evaluated using a TUNEL detection kit (Yuhengbio, Suzhou, Jiangsu, China). In brief, cells were fixed using 4% paraformaldehyde for 30 min and permeabilized with PBS containing 0.1% Triton X-100 for 10 min. Then, a TUNEL reaction mix (50 μ L) was added to the cells in the dark for 1 h, and DAPI was used to counterstain the nuclei. Images were obtained via photography using fluorescence microscopy.

2.11. Statistical analyses

All data are presented as the mean $\pm$ standard deviation (SD) from three independent experiments. SPSS 25.0 statistical software and GraphPad Prism 8.0 were utilized for statistical analyses, and data were

examined using one-way analysis of variance (ANOVA) or Student's *t* test. Differences with $P < 0.05$ were defined as statistically significant.

3. Results

3.1 TRIM32 expression was upregulated in peripheral blood from T2DM patients

ELISA was used to determine the serum TRIM32 concentrations in T2DM, prediabetes, and healthy volunteers. The clinical features of all the volunteers are shown in Table 2 (Online Resource 2). The T2DM group showed significantly increased TRIM32 expression relative to the prediabetes group and the healthy volunteer group, whereas the prediabetes group showed no statistical significance relative to the healthy volunteer group (Fig. 1a).

3.2 High glucose-induced TRIM32 expression in INS-1 cells

To determine the effect of HG on TRIM32 expression, INS-1 cells were treated with glucose at different concentrations (11.1, 22.2, 33.3, 44.4 mM) for 24, 48, and 72 h, and TRIM32 expression was detected using RT-qPCR, western blotting, and immunofluorescence staining. After 24 h of culture, the TRIM32 mRNA and protein levels were increased in a concentration-dependent manner (Fig. 1b). After 48 h of culture, TRIM32 expression in the 33.3 mM glucose-treated group was significantly higher than that in the other groups at the mRNA and protein levels (Fig. 1c). As illustrated in Fig. 1d, exposure to 11.1 mM glucose increased TRIM32 expression in a time-dependent manner. Additionally, immunofluorescence staining showed that TRIM32 was localized in the nucleus of INS-1 cells, and exposure to 33.3 mM glucose for 48 h significantly increased TRIM32 expression compared with that of cells treated with 11.1 mM glucose (Fig. 1e). Therefore, 33.3 mM glucose and 48 h were chosen as the appropriate glucose concentrations and treatment durations, respectively, for subsequent experiments.

3.3 TRIM32 overexpression promoted the inflammatory response in INS-1 cells under HG conditions

To examine the role of TRIM32 in HG-induced inflammatory damage, we induced INS-1 cells to overexpress (oeTRIM32) or silence TRIM32 (shTRIM32). The transfection efficiency was determined using fluorescence microscopy, western blotting, and RT-qPCR (Fig. 2a-c).

Previous studies have demonstrated that NF- κ B and NLRP3 inflammasome activation contribute to pancreatic islet β cell dysfunction [24, 25]. To evaluate the effect of TRIM32 on HG-induced inflammatory damage, INS-1 cells were induced to overexpress or silence TRIM32 and exposed to HG for 48 h, and the protein expression levels of NF- κ B and the NLRP3 inflammasome were estimated using western blotting. The expression of phosphorylated NF- κ B p65, NLRP3, ASC, cleaved caspase-1, IL-1 β , and IL-18 in the HG-treated TRIM32 overexpression group was elevated compared with that in the HG group. TRIM32

silencing had the opposite effects (Fig. 3a, b). Western blot results indicated that TRIM32 overexpression exacerbated HG-induced inflammation.

3.4 TRIM32 overexpression promoted HG-induced pyroptosis in INS-1 cells

TRIM32 overexpression increased the protein expression of the NLRP3 inflammasome, cleaved caspase-1, IL-1 β , and IL-18 in INS-1 cells. Then, we determined the effect of TRIM32 on pyroptosis. As illustrated in Fig. 4, to demonstrate the role of TRIM32 in INS-1-cell pyroptosis, GSDMD-N protein expression, LDH activity, and TUNEL-positive cells were measured. As shown in Fig. 4a, the protein expression of GSDMD-N was significantly increased in HG-treated TRIM32-overexpressing INS-1 cells. LDH activity was measured in cell culture supernatants at the indicated time points. LDH release from TRIM32-overexpressing cells cultured with HG was significantly higher than that from the HG group (Fig. 4b). TUNEL staining results showed that the number of TUNEL-positive cells in the HG-treated TRIM32 overexpression group was significantly higher than that in the HG group (Fig. 4c). However, TRIM32 silencing in INS-1 cells exerted opposite effects.

3.5 TRIM32 overexpression promoted HG-induced inflammation and pyroptosis through the NF- κ B/NLRP3 axis in INS-1 cells

To further determine the correlation between TRIM32 and NF- κ B/NLRP3-mediated inflammation and pyroptosis, TRIM32-overexpressing or TRIM32-silenced INS-1 cells were cultured in HG supplemented with BAY 11-7082. LDH release and TUNEL-positive cells were markedly decreased in the group supplemented with BAY 11-7082 (Fig. 4b and Fig. 5a). NF- κ B blockade reduced the protein expression of GSDMD-N (Fig. 5b), and the protein expression of NLRP3, cleaved caspase-1, ASC, IL-1 β , and IL-18 was also significantly inhibited by BAY 11-7082 (Fig. 5c and d). Collectively, these results demonstrated that TRIM32 overexpression promoted HG-induced inflammation and pyroptosis by regulating the NF- κ B/NLRP signaling pathway.

4. Discussion

This study demonstrated that TRIM32 had a vital role in HG-induced damage in INS-1 cells. In T2DM patient peripheral blood, TRIM32 expression was elevated, and HG stimulated TRIM32 expression in INS-1 cells. TRIM32 overexpression promoted HG-induced inflammatory injury and pyroptosis and the underlying mechanisms were partly through the NF- κ B/NLRP3 signaling pathway.

As the prevalence of T2DM continues to rise, the number of people with diabetic complications will reach an unprecedented level [1]. Thus, the identification of a new therapeutic target is urgently needed. Inflammation, oxidative stress, autophagy, apoptosis, and pyroptosis play essential roles in the

pathogenesis of T2DM and its complications [26–29]. Reduction of inflammation and pyroptosis are potential therapeutic targets for T2DM and its complications. TRIM32, a TRIM protein, has been reported to contribute to insulin resistance in various metabolic conditions, including DM and metabolic syndrome [20]. However, there are no reports on the effect of TRIM32 on pancreatic β -cell dysfunction. In our study, the results indicated that TRIM32 expression was elevated in the peripheral blood of T2DM patients (Fig. 1a), but the mechanism remains unclear. The effect of TRIM32 upregulation in T2DM patient peripheral blood needs to be intensively investigated in the future. Then, we detected the effect of HG on the expression of TRIM32 in INS-1 cells, and the results showed that TRIM32 was highly expressed in HG-treated INS-1 cells (Fig. 1). Recent experiments have demonstrated that TRIM32 is involved in inflammation through its regulation of NF- κ B signaling in rheumatoid arthritis [18] and plays a vital role in HG-evoked inflammatory injury and oxidative stress of podocytes [21]. However, whether TRIM32 is involved in inflammation in INS-1 cells is unknown.

Inflammation was implicated in the occurrence of T2DM and its complications. Markers of inflammation are often elevated in patients suffering from T2DM, and the inhibition of the inflammatory signaling pathway was shown to reduce insulin resistance and improve hyperglycemia in animal models [30]. The NLRP3 inflammasome has been one of the most widely studied inflammasomes over the past decade and is considered to be a therapeutic target for various diseases, including T2DM [31, 32]. The NF- κ B/NLRP3 signaling pathway is involved in the pathogenesis of multiple inflammatory diseases [33, 34]. The present study demonstrated that TRIM32 silencing reduced the HG-induced inflammatory response and cell death. A study about the modulatory mechanisms of TRIM32 in INS-1-cell inflammation is lacking.

Pyroptosis (“fiery death”) is inflammatory caspase-mediated programmed cell death. The canonical inflammasome pathway leads to the activation of the inflammatory caspase-1, which promotes inflammatory cytokine release and pyroptotic cell death [35]. Activated NLRP3 inflammasome catalyzes pro-caspase-1 to form cleaved caspase-1 and then cleaves GSDMD and proinflammatory cytokines to the form pore-forming activity of GSDMD-N and mature cytokines (IL-1 β , IL18). Subsequently, the cell membrane creates pores, resulting in the release of IL-1 β and IL18, and pyroptotic cell death [36]. In this study, the protein levels of IL-1 β , IL18, and GSDMD-N were significantly elevated in the HG-treated TRIM32-overexpressing INS-1 cell group. After cocultures of HG and BAY 11-7082, NF- κ B was inhibited in TRIM32-overexpressing INS-1 cells, the inflammatory response and pyroptosis were attenuated (Fig. 5). It was demonstrated that TRIM32 regulated INS-1 cells’ inflammation and pyroptosis partly through the NF- κ B/NLRP3 signaling pathway.

As previously discussed, TRIM32 plays multiple roles in diverse physiological processes, including apoptosis, inflammation, oxidative stress, autophagy, neuronal development and differentiation, and cell proliferation. The present study indicated that TRIM32 regulated INS-1-cell inflammation and pyroptosis partly through the NF- κ B/NLRP3 signaling pathway. But the regulatory effects of TRIM32 on apoptosis, oxidative stress, and autophagy in INS-1 cells have not been studied, our team will further study it. Furthermore, TRIM32 inhibition attenuates HG-induced apoptosis, oxidative stress, and inflammatory

injury in podocytes, and TRIM32 may also play an important role in the pathogenesis of diabetic nephropathy by mediating podocyte injury [21]. Several TRIM proteins are also involved in the regulation of diabetic complications. For example, TRIM72 overexpression in the myocardium is sufficient to induce diabetic cardiomyopathy by upregulating peroxisome proliferation-activated receptor α and disturbing insulin signal transduction [37]. TRIM63 is related to the pathogenesis of diabetes-associated muscular dystrophy [38]. In addition, TRIM32 plays a protective role against aortic banding-mediated cardiac hypertrophy [39]. Moreover, mutations in TRIM32 cause limb-girdle muscular dystrophy 2H (LGMD2H), and TRIM32 inhibition leads to defective autophagy, accompanied by elevated reactive oxygen species levels [40]. A complex consisting of the ubiquitin ligase TRIM32 and the deubiquitinating enzyme USP7 balances c-Myc ubiquitination and determines the fate of neural stem cells [41]. However, there have been no related studies on the effects of TRIM32 on diabetic cardiomyopathy, diabetes-related muscular atrophy, and diabetic neuropathy. More research is needed, and this will provide a promising therapeutic target for the clinical treatment of T2DM and its complications.

5. Conclusion

The results of this study indicate that TRIM32 regulates the inflammatory response and pyroptosis in INS-1 cells partly via the NF- κ B/NLRP3 signaling pathway. Our study suggests that TRIM32 might be a new target for improving pancreatic β -cell function in T2DM. However, the exact role of TRIM32 in pancreatic β -cell dysfunction requires further investigation further through animal experiments in vivo. The effects of TRIM32 on apoptosis, oxidative stress, and autophagy in pancreatic β -cells also need more research.

Declarations

Author contributions Yanbo Li and Tingting Wan designed the studies. Tingting Wan, Hongjie Wang, Chunxu Wang and Yidan Wang performed the experiments and analyzed the data. The first draft of the manuscript was written by Tingting Wan. Tingting Wan and Xiudan Li revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest All authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval This study was approved by the Ethics Committee of Harbin Medical University and Hulan Red Cross Hospital and strictly followed the principles of the Declaration of Helsinki.

Consent to participate Informed consent was obtained from all individual participants included in the study.

References

1. Sun H, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Chan JCN et al (2021) IDF diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. 109119. Diabetes research and clinical practice <http://doi.org/10.1016/j.diabres.2021.109119>
2. American Diabetes Association (2021) Standards of Medical Care in Diabetes-2021. Classification and Diagnosis of Diabetes. Diabetes Care 44:S15–S33. <http://doi.org/10.2337/dc21-S002>
3. Donath MY, Shoelson SE (2011) Type 2 diabetes as an inflammatory disease. Nat Rev Immunol 11(2):98–107. <http://doi.org/10.1038/nri2925>
4. Böni-Schnetzler M, Meier DT (2019) Islet inflammation in type 2 diabetes. Semin Immunopathol 41(4):501–513. <http://doi.org/10.1007/s00281-019-00745-4>
5. Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA, Kaiser N et al (2002) Glucose-induced beta cell production of IL-1 β contributes to glucotoxicity in human pancreatic islets. J Clin Invest 110(6):851–860. <http://doi.org/10.1172/jci15318>
6. Cookson BT, Brennan MA (2001) Pro-inflammatory programmed cell death. Trends Microbiol 9(3):113–114. [http://doi.org/10.1016/s0966-842x\(00\)01936-3](http://doi.org/10.1016/s0966-842x(00)01936-3)
7. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, Zhuang Y et al (2015) Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature 526(7575):660–665. <http://doi.org/10.1038/nature15514>
8. Hu JJ, Liu X, Xia S, Zhang Z, Zhang Y, Zhao J, Ruan J et al (2020) FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. Nat Immunol 21(5). <http://doi.org/10.1038/s41590-020-0669-6>
9. Bauernfeind F, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, Fernandes-Alnemri T et al (2009) Cutting edge: NF- κ B activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. Journal of immunology (Baltimore, Md: 1950) 12(3): 163–175. <http://doi.org/10.4049/jimmunol.0901363>
10. Hatakeyama S (2017) TRIM Family Proteins: Roles in Autophagy, Immunity, and Carcinogenesis. Trends Biochem Sci 42(4):297–311. <http://doi.org/10.1016/j.tibs.2017.01.002>
11. Jiang W, Cai X, Xu T, Liu K, Yang D, Fan L, Li G et al (2020) Tripartite Motif-Containing 46 Promotes Viability and Inhibits Apoptosis of Osteosarcoma Cells by Activating NF- κ B Signaling Through Ubiquitination of PPAR. Oncol Res 28(4):409–421. <http://doi.org/10.3727/096504020x15868639303417>
12. Xie H, Xie D, Zhang J, Jin W, Li Y, Yao J, Pan Z et al (2020) ROS/NF- κ B Signaling Pathway-Mediated Transcriptional Activation of TRIM37 Promotes HBV-Associated Hepatic Fibrosis. Molecular therapy. Nucleic acids 22:114–123. <http://doi.org/10.1016/j.omtn.2020.08.014>
13. Bai X, Zhang YL, Liu LN (2020) Inhibition of TRIM8 restrains ischaemia-reperfusion-mediated cerebral injury by regulation of NF- κ B activation associated inflammation and apoptosis. Exp Cell

Res 388(2):111818. <http://doi.org/10.1016/j.yexcr.2020.111818>

14. Liu R, Wu H, Song H (2020) Knockdown of TRIM8 Attenuates IL-1 β -induced Inflammatory Response in Osteoarthritis Chondrocytes Through the Inactivation of NF- κ B Pathway. *Cell Transplant* 29:963689720943604. <http://doi.org/10.1177/0963689720943604>
15. Xu H, Yang J, Gao W, Li L, Li P, Zhang L, Gong YN et al (2014) Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature* 513(7517):237–241. <http://doi.org/10.1038/nature13449>
16. Kimura T, Jain A, Choi SW, Mandell MA, Johansen T, Deretic V (2017) TRIM-directed selective autophagy regulates immune activation. *Autophagy* 13(5):989–990. <http://doi.org/10.1080/15548627.2016.1154254>
17. Du Y, Zhang W, Du B, Zang S, Wang X, Mao X, Hu Z (2018) TRIM32 overexpression improves chemoresistance through regulation of mitochondrial function in non-small-cell lung cancers. *OncoTargets and therapy* 11:7841–7852. <http://doi.org/10.2147/ott.s176689>
18. Liang T, Song M, Xu K, Guo C, Xu H, Zhang H, Xu L (2020) TRIM32 promotes inflammatory responses in rheumatoid arthritis fibroblast-like synoviocytes. *Scand J Immunol* 91(6):e12876. <http://doi.org/10.1111/sji.12876>
19. Zhao M, Song K, Hao WZ, Wang LY, Patil G, Li QM, Xu LQ et al (2020) Non-proteolytic ubiquitination of OTULIN regulates NF- κ B signaling pathway. *J Mol Cell Biol* 183(2):787–791. <http://doi.org/10.1093/jmcb/mjz081>
20. Cohen S, Lee D, Zhai B, Gygi SP, Goldberg AL (2014) Trim32 reduces PI3K-Akt-FoxO signaling in muscle atrophy by promoting plakoglobin-PI3K dissociation. *J Cell Biol* 204(5):747–758. <http://doi.org/10.1083/jcb.201304167>
21. Chen Z, Tian L, Wang L, Ma X, Lei F, Chen X, Fu R (2021) TRIM32 Inhibition Attenuates Apoptosis, Oxidative Stress, and Inflammatory Injury in Podocytes Induced by High Glucose by Modulating the Akt/GSK-3 β /Nrf2 Pathway. <http://doi.org/10.1007/s10753-021-01597-7>. *Inflammation*
22. Hohmeier HE, Mulder H, Chen G, Henkel-Rieger R, Prentki M, Newgard CB (2000) Isolation of INS-1-derived cell lines with robust ATP-sensitive K⁺ channel-dependent and -independent glucose-stimulated insulin secretion. *Diabetes* 49(3):424–430. <http://doi.org/10.2337/diabetes.49.3.424>
23. Schwamborn JC, Berezikov E, Knoblich JA (2009) The TRIM-NHL protein TRIM32 activates microRNAs and prevents self-renewal in mouse neural progenitors. *Cell* 136(5):913–925. <http://doi.org/10.1016/j.cell.2008.12.024>
24. Sokolova M, Sahraoui A, Høyem M, Øgaard J, Lien E, Aukrust P, Yndestad A et al (2018) NLRP3 inflammasome mediates oxidative stress-induced pancreatic islet dysfunction. *Am J Physiol Endocrinol Metab* 315(5):E912–E923. <http://doi.org/10.1152/ajpendo.00461.2017>
25. Lee JH, Song MY, Song EK, Kim EK, Moon WS, Han MK, Park JW et al (2009) Overexpression of SIRT1 protects pancreatic beta-cells against cytokine toxicity by suppressing the nuclear factor- κ B signaling pathway. *Diabetes* 58(2):344–351. <http://doi.org/10.2337/db07-1795>

26. Costes S, Bertrand G, Ravier MA (2021) Mechanisms of Beta-Cell Apoptosis in Type 2 Diabetes-Prone Situations and Potential Protection by GLP-1-Based Therapies. *Int J Mol Sci* 22(10). <http://doi.org/10.3390/ijms22105303>
27. Shoelson SE, Lee J, Goldfine AB (2006) Inflammation and insulin resistance. *J Clin Investig* 116(7):1793–1801. <http://doi.org/10.1172/jci29069>
28. Jung HS, Chung KW, Won Kim J, Kim J, Komatsu M, Tanaka K, Nguyen YH et al (2008) Loss of autophagy diminishes pancreatic beta cell mass and function with resultant hyperglycemia. *Cell Metabol* 8(4):318–324. <http://doi.org/10.1016/j.cmet.2008.08.013>
29. Sharma A, Rizky L, Stefanovic N, Tate M, Ritchie RH, Ward KW, de Haan JB (2017) The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) activator dh404 protects against diabetes-induced endothelial dysfunction. *Cardiovasc Diabetol* 16(1):33. <http://doi.org/10.1186/s12933-017-0513-y>
30. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, Ravussin E et al (2011) The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med* 17(2):179–188. <http://doi.org/10.1038/nm.2279>
31. Ambati J, Magagnoli J, Leung H, Wang SB, Andrews CA, Fu D, Pandey A et al (2020) Repurposing anti-inflammasome NRTIs for improving insulin sensitivity and reducing type 2 diabetes development. *Nat Commun* 11(1):4737. <http://doi.org/10.1038/s41467-020-18528-z>
32. Wu AG, Zhou XG, Qiao G, Yu L, Tang Y, Yan L, Qiu WQ et al (2021) Targeting microglial autophagic degradation in NLRP3 inflammasome-mediated neurodegenerative diseases. *Ageing Res Rev* 65:101202. <http://doi.org/10.1016/j.arr.2020.101202>
33. Yi H, Peng R, Zhang LY, Sun Y, Peng HM, Liu HD, Yu LJ et al (2017) LincRNA-Gm4419 knockdown ameliorates NF- κ B/NLRP3 inflammasome-mediated inflammation in diabetic nephropathy. *Cell Death Dis* 8(2):e2583. <http://doi.org/10.1038/cddis.2016.451>
34. Li Y, Xia Y, Yin S, Wan F, Hu J, Kou L, Sun Y et al (2021) Targeting Microglial α -Synuclein/TLRs/NF- κ B/NLRP3 Inflammasome Axis in Parkinson's Disease. *Front Immunol* 12:719807. <http://doi.org/10.3389/fimmu.2021.719807>
35. McKenzie BA, Dixit VM, Power C (2020) Fiery Cell Death: Pyroptosis in the Central Nervous System. *Trends Neurosci* 43(1):55–73. <http://doi.org/10.1016/j.tins.2019.11.005>
36. Xue Y, Enosi Tuipulotu D, Tan WH, Kay C, Man SM (2019) Emerging Activators and Regulators of Inflammasomes and Pyroptosis. *Trends Immunol* 40(11):1035–1052. <http://doi.org/10.1016/j.it.2019.09.005>
37. Liu F, Song R, Feng Y, Guo J, Chen Y, Zhang Y, Chen T et al (2015) Upregulation of MG53 induces diabetic cardiomyopathy through transcriptional activation of peroxisome proliferation-activated receptor α . *Circulation* 131(9):795–804. <http://doi.org/10.1161/circulationaha.114.012285>
38. O'Neill BT, Bhardwaj G, Penniman CM, Krumpoch MT, Suarez Beltran PA, Klaus K, Poro K et al (2019) FoxO Transcription Factors Are Critical Regulators of Diabetes-Related Muscle Atrophy. *Diabetes* 68(3):556–570. <http://doi.org/10.2337/db18-0416>

39. Chen L, Huang J, Ji Y, Zhang X, Wang P, Deng K, Jiang X et al (2016) Tripartite motif 32 prevents pathological cardiac hypertrophy. *Clinical science (London, England: 1979)* 130(10): 813 – 28. <http://doi.org/10.1042/cs20150619>
40. Di Rienzo M, Antonioli M, Fusco C, Liu Y, Mari M, Orhon I, Refolo G et al (2019) Autophagy induction in atrophic muscle cells requires ULK1 activation by TRIM32 through unanchored K63-linked polyubiquitin chains. *Sci Adv* 5(5):eaau8857. <http://doi.org/10.1126/sciadv.aau8857>
41. Nicklas S, Hillje AL, Okawa S, Rudolph IM, Collmann FM, van Wuellen T, Del Sol A et al (2019) A complex of the ubiquitin ligase TRIM32 and the deubiquitinase USP7 balances the level of c-Myc ubiquitination and thereby determines neural stem cell fate specification. *Cell Death Differ* 26(4):728–740. <http://doi.org/10.1038/s41418-018-0144-1>

Figures

Figure 1

TRIM32 was highly expressed in peripheral blood from T2DM patients and high glucose regulated the expression of TRIM32 in INS-1 cells

(a) The level of TRIM32 was measured by ELISA. (b) INS-1 cells were treated with glucose at different concentrations for 24 h, and the mRNA and protein levels of TRIM32 were detected by RT-qPCR and western blotting. (c) INS-1 cells were treated with glucose at different concentrations for 48 h, and the

mRNA and protein levels of TRIM32 were detected by RT-qPCR and western blotting. (d) INS-1 cells were treated with 11.1 mM glucose for 0, 24, 48, and 72 h, and the mRNA and protein levels of TRIM32 were detected by RT-qPCR and western blotting. (e) Representative immunofluorescence staining images. $*P < 0.05$, $**P < 0.01$, ns, nonsignificant. All experiments were repeated at least three times, and the data are presented as the mean $\pm$ SD. HG, high glucose.

Figure 2

Construction of INS-1 cells with TRIM32 overexpression/silencing

(a) Fluorescence microscopy was used to observe the transfection efficiency. (b) Protein levels of TRIM32 were measured by western blotting. (c) mRNA levels of TRIM32 were assayed by RT-qPCR. $*P < 0.05$, $**P < 0.01$. All experiments were repeated at least three times, and the data are presented as the mean $\pm$ SD. oeTRIM32, TRIM32 overexpression; shTRIM32, TRIM32 silencing.

Figure 3

Effect of TRIM32 overexpression on inflammatory response in HG-treated INS-1 cells

(a and b) Protein levels of phosphorylated NF-κB p65, NLRP3, ASC, cleaved caspase-1, IL-1β, and IL-18 were detected by western blot. All experiments were repeated at least three times, and the data are presented as the mean ± SD. * $P < 0.05$ and ** $P < 0.01$. p-NF-κB p65, phosphorylation of NF-κB p65; t-NF-κB p65, total NF-κB p65; cl.casp-1, cleaved caspase-1; oeTRIM32, TRIM32 overexpression; shTRIM32, TRIM32 silencing; HG, high glucose.

Figure 4

TRIM32 overexpression promoted pyroptosis in HG-treated INS-1 cells

(a) The protein level of GSDMD-N was measured by western blotting. The cell death of INS-1 cells was measured by LDH release (b) and TUNEL staining (c). All experiments were repeated at least three times, and the data are presented as the mean $\pm$ SD. * P < 0.05 and ** P < 0.01, ns, nonsignificant. oeTRIM32, TRIM32 overexpression; shTRIM32, TRIM32 silencing; HG, high glucose.

Figure 5

BAY 11-7082 reversed the effects of TRIM32 overexpression on inflammatory response and pyroptosis in INS-1 cells under HG conditions

(a) The cell death of INS-1 cells was measured by TUNEL staining. (b) The protein level of GSDMD-N was measured by western blotting. (c and d) Protein levels of phosphorylation of NF- κ B p65, NLRP3, ASC, cleaved caspase-1, IL-1 β , and IL-18 were detected by western blotting. All experiments were repeated at least three times, and the data are presented as the mean $\pm$ SD. $*P < 0.05$ and $**P < 0.01$. p-NF- κ B p65, phosphorylation of NF- κ B p65; t-NF- κ B p65, total NF- κ B p65; cl.cap-1, cleaved caspase-1; oeTRIM32, TRIM32 overexpression; shTRIM32, TRIM32 silencing; HG, high glucose.

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