JBM J Bone Metab 2024;31(3):209-218
https://doi.org/10.11005/jbm.2024.31.3.209
pISSN 2287-6375 eISSN 2287-7029

Original Article

Thioredoxin Interacting Protein Expressed in Osteoblasts Mediates the Anti-Proliferative Effects of High Glucose and Modulates the Expression of Osteocalcin

Sarah McGarry[1,2], Karen Kover[1,2], Francesco De Luca[1,2]

[1]Division of Endocrinology, Children's Mercy Hospitals, Kansas City, MO;
[2]Department of Pediatrics, University of Missouri-Kansas City-School of Medicine, Kansas City, MO, USA

Corresponding author
Francesco De Luca
Division of Endocrinology, Children's Mercy Hospitals, 2401 Gillham Rd, Kansas City, MO 64108, USA
E-mail: fdeluca59@gmail.com

Received: March 13, 2024
Revised: June 1, 2024
Accepted: June 3, 2024

Background: Hyperglycemia is associated with impaired bone health in patients with diabetes mellitus. Although a direct detrimental effect of hyperglycemia on the bone has been previously reported, the specific molecular mediator(s) responsible for the inhibitory effect of high glucose levels on the bone remains unclear. We hypothesized that thioredoxin-interacting protein (Txnip), an essential mediator of oxidative stress, is such a mediator. **Methods:** We cultured MG-63 cells (immortalized human osteoblasts) with normal or high glucose concentrations and transfected them with scrambled or Txnip-specific small interfering RNA (siRNA). **Results:** High glucose levels increased Txnip expression and reduced MG-63 cell proliferation. The high-glucose level mediated reduction in cell proliferation was prevented in Txnip siRNA-transfected cells. In addition, we demonstrated that silencing Txnip mRNA expression in osteoblasts reduced the expression of the osteocalcin gene. Our results suggest that high glucose levels or silencing of Txnip mRNA expression may induce apoptosis in osteoblasts. **Conclusions:** Our findings indicate that Txnip is an intracellular mediator of the anti-proliferative effects of extracellular high glucose levels on osteoblasts.

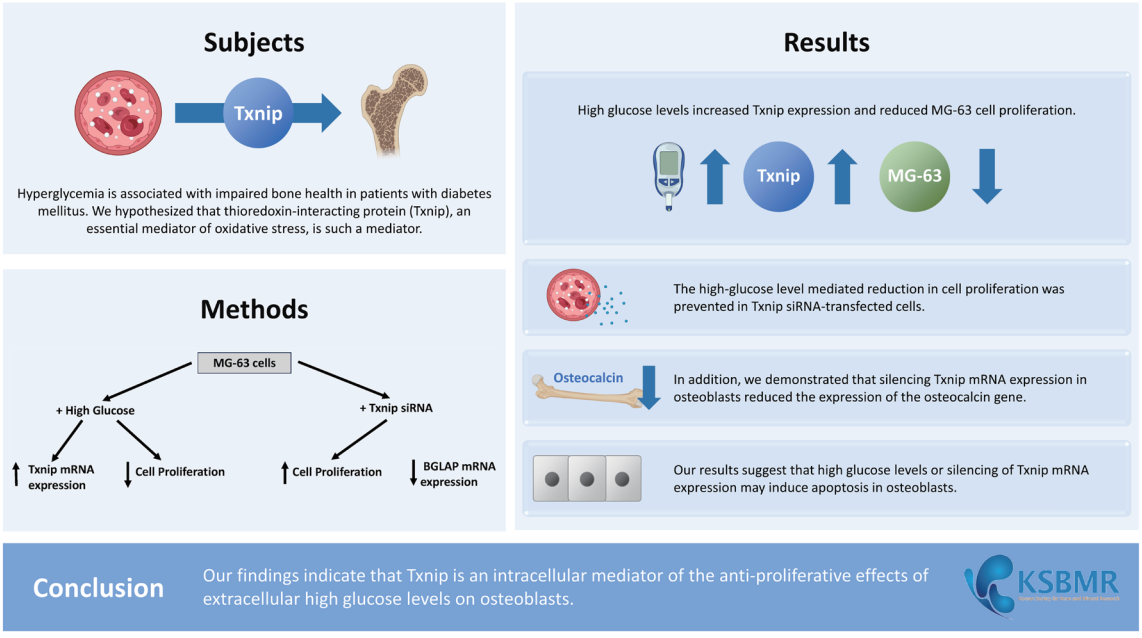
Key Words: Hyperglycemia · Thioredoxin interacting protein · Osteoblasts

INTRODUCTION

Diabetes mellitus (DM) is a very prevalent disease characterized by hyperglycemia, insulin deficiency and/or insulin resistance. Its main long-term complications consist of cardiovascular disease, retinopathy, and chronic kidney insufficiency.[1] In addition, individuals with DM experience an increased risk for bone fractures.[2,3]

In DM, it appears that osteoblast dysfunction is one of the factors contributing to impaired bone quality/mass.[2] An *in vitro* model has been previously utilized to study the effects of hyperglycemia on osteoblasts, culturing osteoblast-like cells in the presence of high glucose in the medium. Published evidence has revealed that high glucose inhibits mineralization and proliferation in MC3T3-E1 cells, an immortalized cell line derived from rat calvaria.[4-8] However, little is

Graphical Abstract



known about any possible molecular mediator directly responsible for the deleterious effect of high glucose on osteoblasts.

Oxidative stress is involved in bone metabolic changes and leads to the formation of high reactive oxygen species (ROS) levels. Excessive ROS activity and defects in the cellular antioxidant system lead to abnormal cellular bone metabolism.[9]

Thioredoxin (TRX)-interacting protein (Txnip), also known as TRX-binding protein-2, is a cytosolic protein that is an essential mediator of oxidative stress and inflammatory response.[10] Txnip is referred to as Txnip because it interacts with TRX, an important intracellular antioxidant protein, and negatively regulates TRX function in many mammalian tissues.[10] TRX, as a thiol oxidoreductase, exerts antioxidant properties through the scavenging of ROS.

In 2003, Kobayashi et al. [11] first reported that Txnip expression level was rapidly elevated following high glucose stimulation in cultured mesangial cells and kidneys of diabetic mice.

In vivo findings showed that the overexpression of TRX in TRX-transgenic mice partially restored the reduced bone mineral density (BMD) and prevented streptozotocin-induced bone formation and osteopenia in diabetes.[12]

Goto-Kakizaki rats, a model of type 2 DM, have significantly reduced bone mass, and the expression of Txnip in their bone tissue is significantly increased.[13]

Yet, there is no evidence that hyperglycemia directly affects the expression of Txnip in bone cells, and that Txnip in turn is responsible of bone cell dysfunction.

In the present study, we have analyzed the effects of high glucose on Txnip expression and function in cultured osteoblast cell line MG-63 (immortalized human osteoblast cell line), which is a prototype of osteoblast phenotype widely utilized in bone research.

METHODS

1. Cell culture and reagents

MG-63 cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) media supplemented with 10% fetal bovine serum (ATCC) and 1% penicillin/streptomycin. Cells were cultured at 37°C in a CO_2 incubator. DMEM high-glucose media was purchased from the ATCC.

https://doi.org/10.11005/jbm.2024.31.3.209

2. Cell proliferation assays

Cell proliferation or viability analysis was carried out by applying the MTT assay. Cells were seeded into 12-well plates (1.5×10^4 cells/well). After 48 or 72 hr of treatment, 10% MTT 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide reagent (5 mg·mL−1) was added and incubated for 4 hr. The supernatant was removed, followed by the addition of 600 µL dimethyl sulfoxide (DMSO) to dissolve formazan crystals. Absorbance was measured at 570 nm. The amount of formazan produced was proportional to the number of viable cells.

3. Flow cytometry analysis

Cells were washed twice with phosphate-buffered saline (PBS), dissociated with 0.05% of trypsin, neutralized in DMEM media, washed twice in PBS and then resuspended in 300 µL Annexin Binding Buffer 5X (Thermo Fisher Scientific, Waltham, MA, USA) diluted 5:1 in PBS. A 3 µL of mouse IgG solution (1 mg/mL) was added and incubated for 5 min on ice. The cells were washed and then resuspended in 300 µL of diluted Annexin Binding Buffer, filtered through a 40 µm strainer, 3 µL (1 mg/mL) of Annexin-V (eBioscience, San Diego, CA, USA) was added and incubated for 15 min at room temperature followed by 3 µL (1 mg/mL) 7-aminoactinomycin D (eBioscience) to assess dead and apoptotic cells. Flow cytometry was performed on a BD LSRFortessa and data analyzed with FlowJo software (Tree Star Inc., Ashland, OR, USA).

4. Small interfering RNA (siRNA) knockdown

siRNAs for Txnip (#AM16708) and the Silencer Select Negative Control #1 siRNA (Scramble, #AM4611) were purchased from Thermo Fisher Scientific as SMARTpools. For siRNA transfections, cells were transfected with oligos using Lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After transfection, efficiency was determined through reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

5. RT-qPCR

Total RNAs were extracted using the RNeasy Kit (Qiagen, Valencia, CA, USA) and RT-qPCR analysis was performed using Bio-Rad MyiQ (Bio-Rad, Hercules, CA, USA). The conditions for RT-qPCR reactions were: one cycle at 95°C for 20 sec followed by 45 cycles at 95°C for 3 sec and annealing at 60°C for 30 sec. Results were normalized to the housekeeping gene 18s ribosomal RNA. Relative expression levels of genes from different groups were calculated with the 2ΔΔCT method and compared with the expression level of appropriate control cells.

6. Statistical analysis

Statistical significance was determined via Student's *t*-test. Results were considered significant when $^{a)}P<0.05$, $^{b)}P<0.01$, or $^{c)}P<0.001$.

RESULTS

1. Effects of high glucose on Txnip expression in MG-63 cells

In MG-63 cells transfected with scramble siRNAs and cultured in the presence of high glucose (25 mM), the expression of Txnip analyzed after 48 hr in culture was significantly greater than in cells transfected with scramble siRNA cultured in the presence of normal glucose concentration (5 mM) (Fig. 1A).

In MG-63 cells transfected with Txnip-specific siRNA, whether cultured in the presence of high glucose or normal glucose, the Txnip expression was significantly reduced when compared to cells transfected with scramble siRNAs and cultured in the presence of high glucose or normal glucose (Fig. 1A).

After 72 hr in culture, the mean Txnip expression in scramble siRNA-transfected MG-63 cells cultured with high glucose was greater than in cells cultured with normal glucose (Fig. 1B). However, this difference did not reach statistical significance.

2. Effects of glucose and Txnip on MG-63 cell proliferation

We then assessed the effects of high glucose on MG-63 cell proliferation by MTT assay. After 48 and 72 hr in culture, MG-63 cells transfected with scramble siRNA and cultured in the presence of high glucose exhibited a significantly decreased cell proliferation compared to cell cultured with normal glucose (Fig. 2). In contrast, at 48 and 72 hr cell proliferation was increased in cells previously transfected with Txnip siRNA and cultured in normal glucose (compared to cells previously transfected with scram-

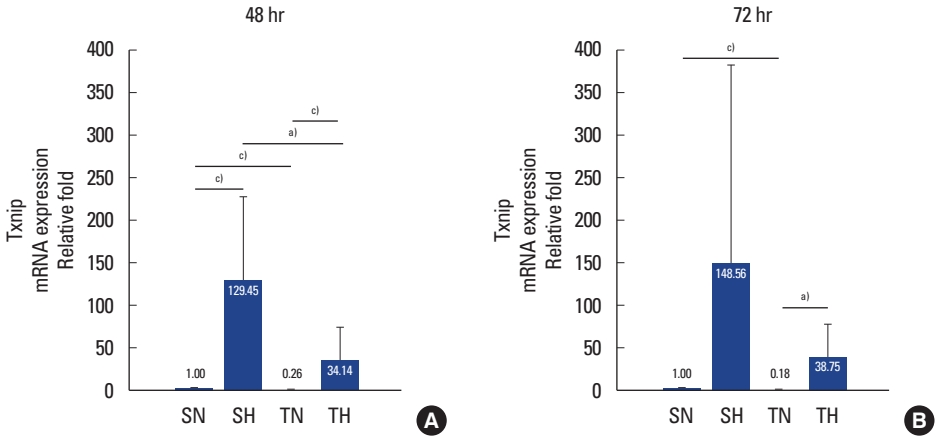


Fig. 1. Effects of high glucose on thioredoxin-interacting protein (Txnip) expression in MG-63 cells. MG-63 cells were maintained in Dulbecco's modified Eagle's medium media supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were cultured in 37°C in a CO_2 incubator. Cells were transfected with a Txnip-specific small interfering RNA (siRNA) or a control, scramble siRNA (Silencer Select Negative Control siRNA), both purchased from Thermo Fisher Scientific (Waltham, MA, USA) as SMARTpools. After transfection, cells were cultured up to 72 hr with normal glucose concentration (5 mM) or high glucose concentration (25 mM) added to the culture medium. After 48 and 72 hr in culture, total RNA was extracted using the RNeasy Kit (Qiagen, Valencia, CA, USA), reverse transcription-quantitative polymerase chain reaction analysis was performed and Txnip mRNA expressed measured after 48 hr (A) and 72 hr (B). Results were normalized to the housekeeping gene 18s ribosomal RNA. SN, cells transfected with scramble siRNA and cultured in normal glucose; SH, cells transfected with scramble siRNA and cultured in high glucose; TN, cells transfected with Txnip-specific siRNA and cultured in normal glucose; TH, cells transfected with Txnip-specific siRNA and cultured in high glucose. [a]$P<0.05$, [c]$P<0.001$.

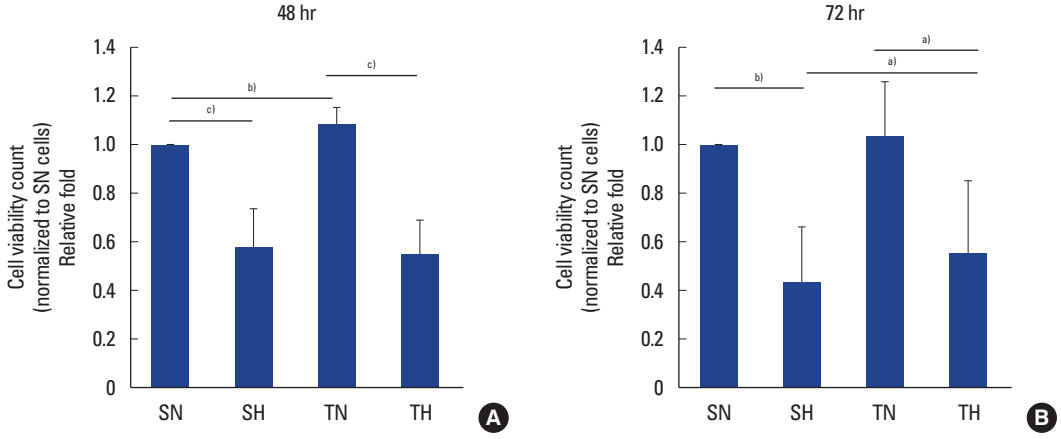


Fig. 2. Effects of high glucose and thioredoxin-interacting protein (Txnip) on MG-63 cell proliferation. MG-63 cells were maintained in Dulbecco's modified Eagle's medium media supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were cultured in 37°C in a CO_2 incubator. Cells were transfected with a Txnip-specific small interfering RNA (siRNA) or a control, scramble siRNA. After transfection, cells were cultured up to 72 hr with normal glucose concentration (5 mM) or high glucose concentration (25 mM) added to the culture medium. Cell proliferation or viability was measured after 48 hr (A) and 72 hr (B) by utilizing the MTT assay. After 48 or 72 hr in culture, 10% MTT was added and incubated for 4 hr. The supernatant was removed, followed by the addition of 600 μL dimethyl sulfoxide to dissolve formazan crystals. Absorbance was measured at 570 nm. The amount of formazan generated was proportional to the number of viable cells. Results are expressed in folds relative to the amount of formazan generated in the culture medium of the SN cells. SN, cells transfected with scramble siRNA and cultured in normal glucose; SH, cells transfected with scramble siRNA and cultured in high glucose; TN, cells transfected with Txnip-specific siRNA and cultured in normal glucose; TH, cells transfected with Txnip-specific siRNA and cultured in high glucose. [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$.

ble siRNA), with the difference being significant only at 48 hr (Fig. 2).

At 72 hr, cell proliferation was increased in Txnip siRNA-transfected MG-63 cells cultured in high glucose compared

https://doi.org/10.11005/jbm.2024.31.3.209

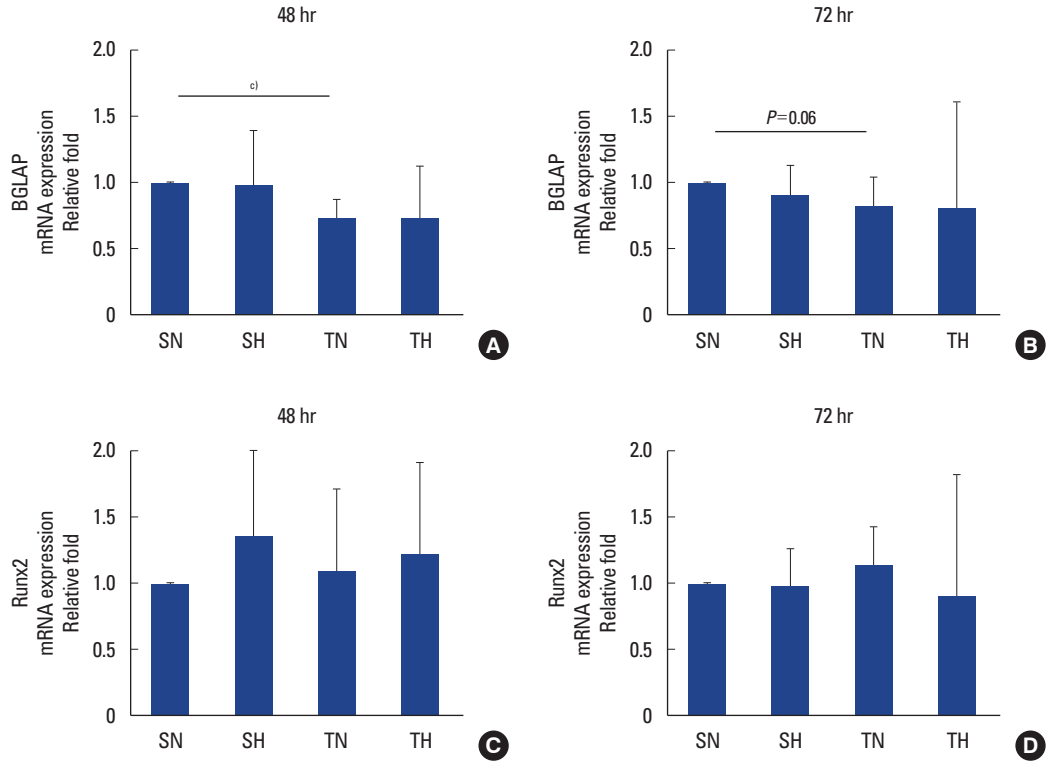


Fig. 3. Effects of thioredoxin-interacting protein (Txnip) on MG-63 cell *BGLAP* and Runx2 mRNA expression. MG-63 cells were maintained in Dulbecco's modified Eagle's medium media supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were cultured in 37°C in a CO_2 incubator. Cells were transfected with a Txnip-specific small interfering RNA (siRNA) or a control, scramble siRNA. After transfection, cells were cultured up to 72 hr with normal glucose concentration (5 mM) or high glucose concentration (25 mM) added to the culture medium. After 48 hr and 72 hr in culture, total RNA was extracted, reverse transcription-quantitative polymerase chain reaction analysis was performed and the mRNA expression of *BGLAP* (A, B) and Runx2 (C, D) (two markers of osteoblast differentiation) measured. Results were normalized to the housekeeping gene 18s ribosomal RNA. SN, cells transfected with scramble siRNA and cultured in normal glucose; SH, cells transfected with scramble siRNA and cultured in high glucose; TN, cells transfected with Txnip-specific siRNA and cultured in normal glucose; TH, cells transfected with Txnip-specific siRNA and cultured in high glucose. [c]$P<0.001$.

to cells transfected with scramble siRNAs and cultured in high glucose (Fig. 2B).

3. Effects of glucose and Txnip on *BGLAP* and Runx2 mRNA expression in MG-63 cells

To study the effects of Txnip on osteoblast differentiation, we analyzed the mRNA expression of *BGLAP* and Runx2 in MG-63 cells transfected with scramble or Txnip siRNA and cultured in the presence of normal or high glucose.

MG-63 transfected with Txnip siRNA (when compared to cells transfected with scramble siRNAs) and cultured in normal glucose exhibited a decreased *BGLAP* expression at 48 and 72 hr (Fig. 3A, B), with the difference being statistically significant at 48 hr (Fig. 3A). High glucose in the culture medium was associated with decreased *BGLAP* expression in MG-63 cells transfected with Txnip siRNA (compared to cells transfected with scramble siRNAs and cultured in high glucose), but this difference did not reach statistical significance. Glucose concentration in the culture medium or type of siRNA did not affect the expression of Runx2 (Fig. 3C, D).

4. Effects of glucose and Txnip on MG-63 cell apoptosis

We then studied the effects of high glucose and Txnip on apoptosis by flow cytometry. When compared to MG-63 cells transfected with scramble siRNA and cultured in the presence of normal glucose, at 48 hr high glucose revealed a two-fold increase in the percentage of cell apoptosis of MG-63 cells transfected with scramble siRNA (Fig. 4A). In addition, cells transfected with Txnip siRNA and cultured with normal glucose exhibited a three-fold mean in-

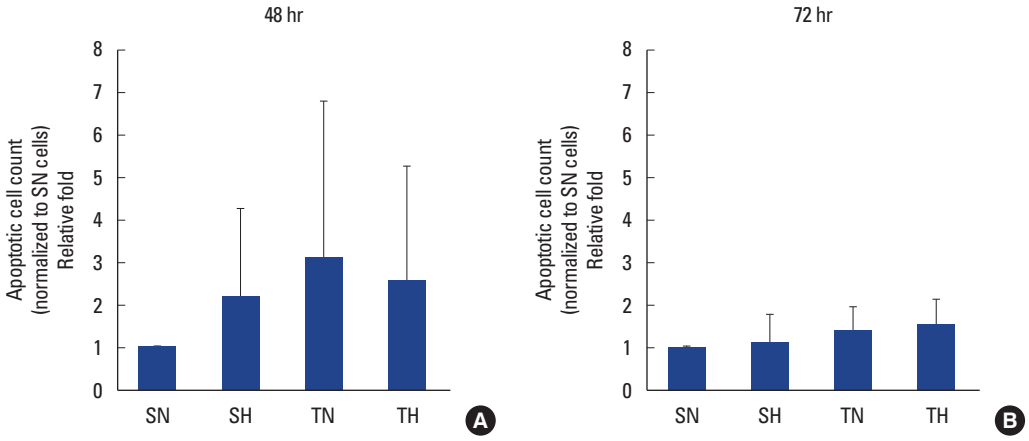


Fig. 4. Effects of high glucose and thioredoxin-interacting protein (Txnip) on MG-63 cell apoptosis. Cell apoptosis was analyzed after 48 hr (A) and 72 hr (B) by using flow cytometry. After 48 hr and 72 hr in culture, cells were washed twice with phosphate-buffered saline (PBS), dissociated with 0.05% of trypsin, neutralized in Dulbecco's modified Eagle's medium media, washed twice in PBS and then resuspended in 300 μL Annexin Binding Buffer 5X diluted 5:1 in PBS. 3 μL of mouse immunoglobulin G solution (1 mg/mL) was added and incubated for 5 min on ice. The cells were washed and then resuspended in 300 μL of diluted Annexin Binding Buffer, filtered through a 40 μm strainer, 3 μL (1 mg/mL) of Annexin V was added and incubated for 15 min at room temperature followed by 3 μL (1 mg/mL) 7-aminoactinomycin D to assess apoptotic cells. Flow cytometry was performed on a BD LSRFortessa and data analyzed with FlowJo software (Tree Star Inc., Ashland, OR, USA). siRNA, small interfering RNA; SN, cells transfected with scramble siRNA and cultured in normal glucose; SH, cells transfected with scramble siRNA and cultured in high glucose; TN, cells transfected with Txnip-specific siRNA and cultured in normal glucose; TH, cells transfected with Txnip-specific siRNA and cultured in high glucose.

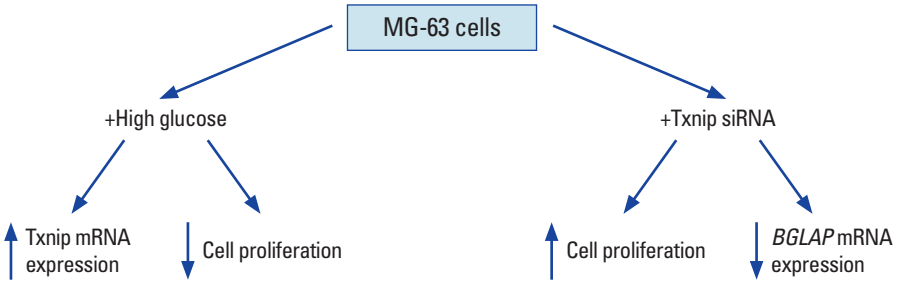


Fig. 5. Schematic summary of our findings. MG-63 cells cultured in the presence of high glucose (25 mM) in the medium exhibited increased thioredoxin-interacting protein (Txnip) mRNA expression and decreased cell proliferation. After transfection with Txnip small interfering RNA (siRNA), decreased Txnip mRNA expression resulted in increased cell proliferation and decreased *BGLAP* mRNA expression.

crease in apoptosis, compared to cells transfected with scramble siRNA cultured in normal glucose (Fig. 4A).

At 72 hr in culture, high glucose or silenced Txnip expression did not induce any obvious change in percentage of cell apoptosis compared to cells transfected with scramble siRNA and cultured in normal glucose (Fig. 4B).

The significant effects of high glucose and Txnip silenced expression in osteoblasts are summarized in Figure 5.

DISCUSSION

Several lines of evidence indicate that DM is associated with impaired bone health. The prevalence of osteoporotic fractures is increased in patients with type 1 DM or type 2 DM.[14] While some studies have suggested a reduced BMD in subjects with DM,[15,16] others have found no change or even increased BMD, implying bone fragility/decreased bone quality as a primary causative mechanism of increased predisposition to bone fractures.[17-19] Hyperglycemia and hypoglycemia, which are both common metabolic derangements in DM, have been associated with increased fracture risk.[20-24]

Prior studies have implied a direct detrimental role of hyperglycemia on osteoblast proliferation. High concen-

https://doi.org/10.11005/jbm.2024.31.3.209

tration of glucose in the culture media inhibits proliferation of osteoblast-like cells [25,26] as well as of mesenchymal stem cells.[27]

We have hypothesized that Txnip is a mediator of the direct effects of hyperglycemia on osteoblasts. Txnip is a critical mediator of intracellular oxidative stress [28-30]; it functions by interacting and neutralizing TRX, an important intracellular antioxidant protein. It has been demonstrated that Txnip expression is consistently increased in skeletal muscle cells of diabetic and prediabetic patents.[31] High glucose enhances Txnip expression in cultured renal proximal tubule cell lines,[31] in glomerular mesangial cells,[32] and in breast-cancer-derived cells.[33] In addition, Txnip is expressed in bone.[28,34,35]

To test our hypothesis, we utilized MG-63 cells, an immortalized human osteoblast cell line.[25] In our study, we have demonstrated that high glucose in the culture media inhibits MG-63 cell proliferation and increases Txnip expression. The increased expression of Txnip has a causative role in the high glucose-mediated decreased cell proliferation. Indeed, Txnip silenced expression by siRNA in MG-63 cells prevents the inhibitory effects of high glucose on proliferation.

An anti-proliferative role of Txnip has also been shown in other cells. HTR-8/SVneo cells (a human trophoblast cell line) treated with high glucose exhibit reduced proliferation, with the knockdown of Txnip reversing the effects of high glucose on HTR-8/Svneo cells.[36] The reduced proliferation induced by interleukin-1β in cultured articular chondrocytes is prevented by silencing of Txnip via siRNA.[37]

In our study, we have also demonstrated a reduced expression of *BGLAP* (the gene encoding osteocalcin) in MG-63 cells transfected with Txnip siRNA, which suggests a regulatory role of Txnip on osteocalcin expression in osteoblasts. Osteocalcin, or bone γ-carboxyglutamic acid protein, is a factor expressed and secreted only by osteoblasts.[36] It is the most abundant noncollagenous protein present in bone. Osteocalcin is initially synthesized as a prohormone (95 amino acids) and then cleaved to form the mature peptide. In humans, the mature peptide has 49 amino acids and is carboxylated at positions 17, 21, and 24. Carboxylation increases the affinity of osteocalcin to the bone extracellular matrix. Osteoclast resorption, on the other hand, creates an acidic environment where osteocalcin is decarboxylated. Undercarboxylated osteocalcin has lower affinity to bone matrix and is released into the bloodstream and it can function as a hormone.[38] Evidence provided by studies on osteocalcin knock-out mice revealed a role, possibly marginal, for osteocalcin in regulating bone formation and mineralization.[39] In contrast, more recent evidence suggests a complex regulatory role for the systemic form of Osteocalcin released from bone into circulation on energy expenditure, glucose homeostasis, insulin secretion, and brain function.[40] Our finding of a modulatory effect of Txnip on *BGLAP*-osteocalcin expression is novel and suggests a possible, indirect, contribution of Txnip in regulating a variety of metabolic pathways in mammals. A previous study has described an increased expression of osteocalcin in osteoblasts transfected with Txnip siRNA,[34] which contradicts our finding. One possible explanation is the different cell lines used (MG-63 in our study, human fetal osteoblast cell line in the previous study).

Our experiments on the effect of high glucose and Txnip on cell apoptosis suggested (after 48 hr in culture) a trend of increased apoptosis induced by high glucose or by the silencing of Txnip expression in MG-63 cells. However, none of these effects was significant.

Previous reports suggest a stimulatory effect of high glucose on cell apoptosis in other cell types,[36,41] with this effect being prevented by the knockdown of Txnip expression. In another study on human osteoblasts, high glucose increased Caspase 3 activity (with Caspase 3 being a marker of apoptosis). However, this effect was observed after 7 days in culture.[42]

In conclusion, our study has demonstrated that high glucose induces reduced proliferation of cultured immortalized human osteoblast cells, and that this effect is mediated by the increased expression and activity of intracellular Txnip. Furthermore, we have shown that Txnip in osteoblasts modulates the expression of the osteocalcin gene, with the silencing of Txnip expression resulting in reduced osteocalcin gene expression. The trend of increased apoptosis in osteoblasts cultured in high glucose or after transfection with Txnip siRNA is not significant.

DECLARATIONS

Funding

The authors received no external financial support for this article.

Ethics approval and consent to participate

This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

ORCID

Sarah McGarry *https://orcid.org/0000-0001-9362-7053*
Karen Kover *https://orcid.org/0000-0001-9866-1904*
Francesco De Luca *https://orcid.org/0000-0002-7186-2316*

REFERENCES

1. Wu Y, Ding Y, Tanaka Y, et al. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. Int J Med Sci 2014;11:1185-200. https://doi.org/10.7150/ijms.10001.
2. Kanazawa I, Sugimoto T. Diabetes mellitus-induced bone fragility. Intern Med 2018;57:2773-85. https://doi.org/10.2169/internalmedicine.0905-18.
3. Farr JN, Drake MT, Amin S, et al. In vivo assessment of bone quality in postmenopausal women with type 2 diabetes. J Bone Miner Res 2014;29:787-95. https://doi.org/10.1002/jbmr.2106.
4. Dong K, Hao P, Xu S, et al. Alpha-lipoic acid alleviates high-glucose suppressed osteogenic differentiation of MC3T3-E1 cells via antioxidant effect and PI3K/Akt signaling pathway. Cell Physiol Biochem 2017; 42:1897-906. https://doi.org/10.1159/000479605.
5. Ogawa N, Yamaguchi T, Yano S, et al. The combination of high glucose and advanced glycation end-products (AGEs) inhibits the mineralization of osteoblastic MC3T3-E1 cells through glucose-induced increase in the receptor for AGEs. Horm Metab Res 2007;39:871-5. https://doi.org/10.1055/s-2007-991157.
6. Medeiros C, Wallace JM. High glucose-induced inhibition of osteoblast like MC3T3-E1 differentiation promotes mitochondrial perturbations. PLoS One 2022;17:e0270001. https://doi.org/10.1371/journal.pone.0270001.
7. Yang J, Ma C, Zhang M. High glucose inhibits osteogenic differentiation and proliferation of MC3T3-E1 cells by regulating P2X7. Mol Med Rep 2019;20:5084-90. https://doi.org/10.3892/mmr.2019.10790.
8. Cunha JS, Ferreira VM, Maquigussa E, et al. Effects of high glucose and high insulin concentrations on osteoblast function in vitro. Cell Tissue Res 2014;358:249-56. https://doi.org/10.1007/s00441-014-1913-x.
9. Manolagas SC. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. Endocr Rev 2010;31:266-300. https://doi.org/10.1210/er.2009-0024.
10. Nishinaka Y, Masutani H, Oka S, et al. Importin alpha1 (Rch1) mediates nuclear translocation of thioredoxin-binding protein-2/vitamin D(3)-up-regulated protein 1. J Biol Chem 2004;279:37559-65. https://doi.org/10.1074/jbc.M405473200.
11. Kobayashi T, Uehara S, Ikeda T, et al. Vitamin D3 up-regulated protein-1 regulates collagen expression in mesangial cells. Kidney Int 2003;64:1632-42. https://doi.org/10.1046/j.1523-1755.2003.00263.x.
12. Hamada Y, Fujii H, Kitazawa R, et al. Thioredoxin-1 overexpression in transgenic mice attenuates streptozotocin-induced diabetic osteopenia: a novel role of oxidative stress and therapeutic implications. Bone 2009;44:936-41. https://doi.org/10.1016/j.bone.2008.12.011.
13. Liang Y, Liu Y, Lai W, et al. 1,25-Dihydroxy vitamin D3 treatment attenuates osteopenia, and improves bone muscle quality in Goto-Kakizaki type 2 diabetes model rats. Endocrine 2019;64:184-95. https://doi.org/10.1007/s12020-019-01857-5.
14. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes--a meta-analysis. Osteoporos Int 2007;18:427-44. https://doi.org/10.1007/s00198-006-0253-4.
15. Faienza MF, Pontrelli P, Brunetti G. Type 2 diabetes and bone fragility in children and adults. World J Diabetes 2022;13:900-11. https://doi.org/10.4239/wjd.v13.i11.900.
16. Kurra S, Siris E. Diabetes and bone health: the relationship between diabetes and osteoporosis-associated fractures. Diabetes Metab Res Rev 2011;27:430-5. https://doi.org/10.1002/dmrr.1197.
17. Bonds DE, Larson JC, Schwartz AV, et al. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. J Clin Endocrinol Metab 2006;91:3404-10. https://doi.org/10.1210/jc.2006-0614.
18. Ma L, Oei L, Jiang L, et al. Association between bone mineral density and type 2 diabetes mellitus: a meta-analysis

https://doi.org/10.11005/jbm.2024.31.3.209

of observational studies. Eur J Epidemiol 2012;27:319-32. https://doi.org/10.1007/s10654-012-9674-x.
19. Leslie WD, Aubry-Rozier B, Lamy O, et al. TBS (trabecular bone score) and diabetes-related fracture risk. J Clin Endocrinol Metab 2013;98:602-9. https://doi.org/10.1210/jc.2012-3118.
20. Ivers RQ, Cumming RG, Mitchell P, et al. Diabetes and risk of fracture: the Blue Mountains Eye Study. Diabetes Care 2001;24:1198-203. https://doi.org/10.2337/diacare.24.7.1198.
21. Oei L, Zillikens MC, Dehghan A, et al. High bone mineral density and fracture risk in type 2 diabetes as skeletal complications of inadequate glucose control: the Rotterdam Study. Diabetes Care 2013;36:1619-28. https://doi.org/10.2337/dc12-1188.
22. Puar TH, Khoo JJ, Cho LW, et al. Association between glycemic control and hip fracture. J Am Geriatr Soc 2012;60:1493-7. https://doi.org/10.1111/j.1532-5415.2012.04052.x.
23. Li CI, Liu CS, Lin WY, et al. Glycated hemoglobin level and risk of hip fracture in older people with type 2 diabetes: a competing risk analysis of Taiwan diabetes cohort study. J Bone Miner Res 2015;30: 1338-46. https://doi.org/10.1002/jbmr.2462.
24. Dufour AB, Kiel DP, Williams SA, et al. Risk factors for incident fracture in older adults with type 2 diabetes: the framingham heart study. Diabetes Care 2021;44:1547-55. https://doi.org/10.2337/dc20-3150.
25. Terada M, Inaba M, Yano Y, et al. Growth-inhibitory effect of a high glucose concentration on osteoblast-like cells. Bone 1998;22:17-23. https://doi.org/10.1016/s8756-3282(97)00220-2.
26. Zhang P, Liao J, Wang X, et al. High glucose promotes apoptosis and autophagy of MC3T3-E1 osteoblasts. Arch Med Sci 2023;19:138-50. https://doi.org/10.5114/aoms.2020.101307.
27. Li YM, Schilling T, Benisch P, et al. Effects of high glucose on mesenchymal stem cell proliferation and differentiation. Biochem Biophys Res Commun 2007;363:209-15. https://doi.org/10.1016/j.bbrc.2007.08.161.
28. Jiang N, Liu J, Guan C, et al. Thioredoxin-interacting protein: a new therapeutic target in bone metabolism disorders? Front Immunol 2022;13:955128. https://doi.org/10.3389/fimmu.2022.955128.
29. Minn AH, Hafele C, Shalev A. Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis. Endocrinology 2005;146:2397-405. https://doi.org/10.1210/en.2004-1378.
30. Choi EH, Park SJ. TXNIP: a key protein in the cellular stress response pathway and a potential therapeutic target. Exp Mol Med 2023;55:1348-56. https://doi.org/10.1038/s12276-023-01019-8.
31. Parikh H, Carlsson E, Chutkow WA, et al. TXNIP regulates peripheral glucose metabolism in humans. PLoS Med 2007;4:e158. https://doi.org/10.1371/journal.pmed.0040158.
32. Xu W, Wang L, Li J, et al. TXNIP mediated the oxidative stress response in glomerular mesangial cells partially through AMPK pathway. Biomed Pharmacother 2018;107:785-92. https://doi.org/10.1016/j.biopha.2018.08.067.
33. Turturro F, Von Burton G, Friday E. Hyperglycemia-induced thioredoxin-interacting protein expression differs in breast cancer-derived cells and regulates paclitaxel IC50. Clin Cancer Res 2007;13:3724-30. https://doi.org/10.1158/1078-0432.Ccr-07-0244.
34. Lekva T, Ueland T, Bøyum H, et al. TXNIP is highly regulated in bone biopsies from patients with endogenous Cushing's syndrome and related to bone turnover. Eur J Endocrinol 2012;166:1039-48. https://doi.org/10.1530/eje-11-1082.
35. Mo Y, Lai W, Zhong Y, et al. TXNIP contributes to bone loss via promoting the mitochondrial oxidative phosphorylation during glucocorticoid-induced osteoporosis. Life Sci 2021;266:118938. https://doi.org/10.1016/j.lfs.2020.118938.
36. Gu Y, Cao M, Chen Y, et al. Knockdown of TXNIP alleviates gestational diabetes mellitus by activating autophagy to regulate cell proliferation and apoptosis in high glucose-treated trophoblasts. Reprod Biol 2024;24:100841. https://doi.org/10.1016/j.repbio.2023.100841.
37. Xu S, Lin W, Lin W, et al. Blocking TXNIP reduced IL-1β Induced chondrocyte cell inflammation. Cell Mol Biol (Noisy-le-grand) 2023;69:174-9. https://doi.org/10.14715/cmb/2023.69.15.30.
38. Wang JS, Mazur CM, Wein MN. Sclerostin and osteocalcin: candidate bone-produced hormones. Front Endocrinol (Lausanne) 2021;12:584147. https://doi.org/10.3389/fendo.2021.584147.
39. Ducy P, Desbois C, Boyce B, et al. Increased bone formation in osteocalcin-deficient mice. Nature 1996;382:448-52. https://doi.org/10.1038/382448a0.
40. Karsenty G. Osteocalcin: a multifaceted bone-derived hormone. Annu Rev Nutr 2023;43:55-71. https://doi.org/10.1146/annurev-nutr-061121-091348.

41. Kover K, Yan Y, Tong PY, et al. Osteocalcin protects pancreatic beta cell function and survival under high glucose conditions. Biochem Biophys Res Commun 2015;462:21-6. https://doi.org/10.1016/j.bbrc.2015.04.095.
42. Shahen VA, Schindeler A, Rybchyn MS, et al. Rescue of high glucose impairment of cultured human osteoblasts using cinacalcet and parathyroid hormone. Calcif Tissue Int 2023;112:452-62. https://doi.org/10.1007/s00223-023-01062-7.

https://doi.org/10.11005/jbm.2024.31.3.209