Research Article

The Chondroprotective Role of TMF in PGE\textsubscript{2}-Induced Apoptosis Associating with Endoplasmic Reticulum Stress

Jianqiong Yang,\textsuperscript{1} Haiqing Liu,\textsuperscript{2} Linfu Li,\textsuperscript{2} Hai Liu,\textsuperscript{2} Weimei Shi,\textsuperscript{2} and Longhuo Wu\textsuperscript{2}

\textsuperscript{1}Department of Clinical Research Center, The First Affiliated Hospital of Gannan Medical University, Ganzhou 341000, China
\textsuperscript{2}College of Pharmacy, Gannan Medical University, Ganzhou 341000, China

Correspondence should be addressed to Longhuo Wu; longhwu@hotmail.com

Received 28 May 2015; Revised 21 July 2015; Accepted 25 August 2015

Academic Editor: Luciana Dini

Copyright © 2015 Jianqiong Yang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Endoplasmic reticulum stress (ERS) has been demonstrated to exhibit a critical role in osteoarthritic chondrocytes. Whether 5,7,3',4'-tetramethoxyflavone (TMF) plays the chondroprotective role in inhibition of PGE\textsubscript{2}-induced chondrocytes apoptosis associating with ERS has not been reported. To investigate this, the activation of PERK, ATF6, and IRE1 signaling pathways in ERS in chondrocytes pretreated with PGE\textsubscript{2} was studied. By treatment with PGE\textsubscript{2}, the chondrocytes apoptosis was significantly increased, the proapoptotic CHOP and JNK were upregulated, the prosurvival GRP78 and XBP1 were downregulated, and GSK-3\textsubscript{\beta} was also upregulated. However, TMF exhibited the effectively protective functions via counteracting these detrimental effects of PGE\textsubscript{2}. Finally, the inflammatory cytokine PGE\textsubscript{2} can activate ERS signaling and promote chondrocytes apoptosis, which might be associated with upregulation of GSK-3\textsubscript{\beta}. TMF exhibits a chondroprotective role in inhibiting PGE\textsubscript{2}-induced ERS and GSK-3\textsubscript{\beta}.

1. Introduction

Endoplasmic reticulum stress (ERS) might be stimulated by a dysfunctional activity of ER dealing with the misfolded or unfolded proteins [1]. The resident transmembrane proteins (PERK, ATF6, and IRE1) are the three ER sensors involved in unfolded protein response (UPR), which functions to maintain the homeostasis of ER by attenuating protein translation, reducing the load of newly synthesized protein, degrading misfolded or unfolded protein, and upregulating chaperones, such as Grp78 [2]. However, if the protective responses fail and the detrimental factors and ERS persist, cells are indexed to switch from prosurvival to proapoptosis [3], leading to enhanced expression of CHOP and upregulation of ER-related caspase-12.

Recently, it has been demonstrated that ERS is sensitively implicated in chondrocytes and associated with human osteoarthritis cartilage [4]. PERK and ATF6 signaling pathways activate the downstream factor CHOP to downregulate the expression of Bcl-2, an antiapoptotic protein, and contribute to programmed cell death [5], which has been comprehensively reviewed [6]. However, XBPI and the chaperone GRP78 exhibit prosurvival functions to promote protein processing and provision during ERS. GSK-3\textsubscript{\beta}, which is a multifunctional Ser/Thr kinase, has been identified to be a major regulator of inflammatory responses and be involved in ERS [7]. GSK-3\textsubscript{\beta} has been showed to regulate ERS-activated CHOP expression. Inhibition of GSK-3\textsubscript{\beta} can significantly downregulate the expression of CHOP in neuronal cells [8]. In our previous studies, we found that PGE\textsubscript{2} could upregulate the expression of GSK-3\textsubscript{\beta} and this could be significantly inhibited by 5,7,3',4'-tetramethoxyflavone (TMF), which is one of active polymethoxyflavone compounds isolated from Murraya exotica [9]. In this paper, we will discuss whether PGE\textsubscript{2} can induce activation of ERS and GSK-3\textsubscript{\beta} in chondrocytes and the protection role of TMF.

2. Materials and Methods

2.1. Materials. TMF has been isolated from the leaves of M. exotica and purified by repeated column chromatography and recrystallization in acetone. The purity was found to be more than 98%. Fetal bovine serum (FBS), Dulbecco’s modified Eagle’s minimum essential medium (DMEM)
GSK-3 continued to digest at 37°C cartilage for 30 min. Then, 0.2% collagenase II was used to remove other tissues and cells, and the peroxidase-conjugated secondary antibody was employed to fix the cells. A tunnel and DAPI staining kit was used for determining the cell apoptosis. A FITC-annexin V/PI double-fluorescence labeling kit was used for detection of the genes expression of CHOP, GRP78, Bak, and Bak. The primer sequences were listed in Table 1.

| Genes  | Forward  | Reverse  | Reference |
|--------|----------|----------|-----------|
| CHOP   | 5'-CAGAACCAGCAGAGGTCACA-3' | 5'-AGCTTGAGCCTTACCTTTTCC-3' | Reference [10] |
| GRP78  | 5'-GCTGACTCGAATCCAAAG-3' | 5'-TTGTGCAGGGGCTTTACC-3' | Reference [11] |
| XBPI   | 5'-AGTTAAGACACGCTTGGGAT-3' | 5'-AAAGATGGTTCTGGGAGGTGAC-3' | Reference [12] |
| Bax    | 5'-TTCCCAGGAGGCTTTT-3' | 5'-CGGCCGAGGTTAGTTC-3' | Reference [13] |
| Bak    | 5'-GTCCGGAGCAGCTAGAGACAT-3' | 5'-AGCATCAATTTCATCGCCTATCT-3' | Reference [14] |
| GAPDH  | 5'-CAGTGCGAAATGTGAGATTC-3' | 5'-AATTTGCGG-3' | Reference [15] |

2.2. Primary Cell Cultures. The ethical agreement has been granted by the Institutional Animal Care and Use Committee of Gannan Medical University. The cartilage was obtained from the knee joint of four-week-old rats under sterile conditions. In order to remove other tissues and cells, 0.25% pancreatic enzymes have been used for digestion of the cartilage for 30 min. Then, 0.2% collagenase II was used to continue to digest at 37°C for 4 h. Cells were cultured at 37°C with 5% CO2 in 10% FBS of the medium, including DMEM (low glucose), 100 μL/mL penicillin, and 100 mg/mL streptomycin. The primary chondrocytes were used for the assays.

2.3. Tunnel and DAPI Staining Assay. Chondrocytes were cultured and administrated with 1 μM PGE2 and TMF. After 48 h treatment, cells were harvested. 4% paraformaldehyde was employed to fix the cells. A tunnel and DAPI staining kit (Abcam, USA) was used for determining the cell apoptosis. A Leica DM3000 microscope was employed for all fluorescent images. And DFC 420 camera (Leica, Germany) functions to take the photos.

2.4. Quantitative Analysis of Apoptosis Cells. The FITC-annexin V/PI double-fluorescence labeling kit was used for analyzing the cell apoptosis by flow cytometry. The procedures were strictly performed under the recommendation of the kit (Nanjing KeyGEN Biological Technology Development Co., Ltd., Nanjing, China). After treatment with 1 μM PGE2 and TMF, cells (1 × 10⁶/mL) were centrifuged and incubated to be stained by FITC-annexin V and PI. The flow cytometer (FACSCalibur BD, San Jose, CA) was employed for measurement of apoptosis.

2.5. Gene Expression Analysis. The easy-spin total RNA extraction kit (iNtRON Biotechnology, Seoul, Korea) was used for extraction of the total RNA from chondrocytes. Following standard protocols, M-MLV (Promega, USA) was employed to reverse-transcribe total RNA to cDNA, that is, using 2 μg of total RNA to synthesize the first strand of cDNA for each sample. EzOomics SYBR qPCR kits purchased from Biomics in a Mastercycler (Eppendorf) were used for detection of the genes expression of CHOP, GRP78, XBPI, Bax, and Bak. The primer sequences were listed in Table 1. Amplification procedure was 94°C for 5 min, followed by 30 cycles at 94°C for 30 s, 56°C for 45 s, 72°C for 45 s, and finally 72°C for 10 min. The procedures of PCR assay were made by employing the iCycler iQ real time PCR system (Bio-Rad).

The PCR assays were set to perform with four duplications. GAPDH was used as an internal standard control. Primer and template designs following the same criteria for each target, primers, and Mg2+ concentrations had been optimized to render efficiency for each target near one per assumption underlying the 2−ΔΔCT method [15].

2.6. Western Blot Analysis. After lysis in the buffer (2% SDS, 10% glycerol, 10 mmol/L Tris, pH 6.8, and 100 mmol/L DTT), cells were performed with immunoblotting. Using bovine serum albumin as a standard, the protein concentrations were determined by employing a BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). After being mixed with gel loading buffer (50 mmol/L Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, and 0.1% bromophenol blue) and denatured for 5 min, each sample (50 μg) was electrophoresed on 10% SDS-PAGE gel for GRP78, PERK, p-PERK, eIF2α, ATF6, IRE1α, XBPIs, CHOP, JNK, GSK-3β, Bax, and Bak, respectively. Proteins were separated and Western-blotted onto a transfer membranes made of polyvinylidene difluoride (PVDF). Tris-buffered saline (TBS) containing 5% nonfat milk was used to block the blots for 1 h. Then, these blots were incubated with GRP78, PERK, p-PERK, eIF2α, ATF6, IRE1α, XBPIs, CHOP, JNK, GSK-3β, Bax, and Bak at
Evidence-Based Complementary and Alternative Medicine

Figure 1: TMF inhibited chondrocytes apoptosis induced by 1 µM PGE2. The normal chondrocytes were incubated for 48 h. In the control group (a), chondrocytes were incubated with no medicines. Model group (b) was the normal chondrocytes incubated with 1 µM PGE2. Figures (c)–(e) were the groups incubated with 1 µM PGE2 and 5, 10, and 20 µg/mL TMF, respectively. Figure (f) was the summarized data obtained from tunnel staining to indicate the rate of apoptosis cells. Data were expressed by mean ± SD of 4 replicates. *p < 0.05 as compared with control.

4 ºC over night, respectively. Rinsed by PBS, these blots were then incubated with HRP-conjugated goat anti-rat IgG for 1 h. After washing, the blots were set for detection. A super enhanced chemiluminescence detection kit (Applygen Technologies Inc., Beijing, China) was employed, and the protein bands were visualized after exposure of the membranes to Kodak film (USA). GAPDH was used as the internal control in all Western blot analyses.

2.7. Statistical Analysis. All data were presented as mean ± standard deviation (SD). Gene expression data were statistically analyzed by a paired t-test. Differences were considered significant at p < 0.05.

3. Results

3.1. TMF Decreased PGE2-Induced Apoptosis Ratio of Chondrocytes In Vitro. To observe the effect of TMF on chondrocytes cell death, 1 µM PGE2 and TMF (5, 10, and 20 µg/mL) were added to the cultured medium for 48 h. Cell apoptosis was analyzed by tunnel staining and flow cytometry using the FITC-annexin V/PI double staining. As showed in Figures 1 and 2, PGE2 could significantly promote chondrocytes apoptosis, and TMF exhibited effectively chondroprotective activity. In the model group, the chondrocytes apoptosis rates were 10.14 ± 1.36% in tunnel staining (Figure 1) and 14.50 ± 1.89% in FITC-annexin V/PI double staining (Figure 2). In contrast, at the dose of 20 µg/mL of TMF, they showed that the chondrocytes apoptosis rates are almost as moderate as those in the control group.

3.2. TMF Downregulated the Expressions of CHOP, Bax, and Bak but Upregulated GRP78 and XBPI Genes. PGE2, produced by COX-2, is a proinflammatory cytokine. To determine whether PGE2 exhibits its inflammatory role in inducing ERS, changes in CHOP, Bax, Bak, GRP78, and XBPI mRNA expressions were assessed using qRT-PCR (Figure 3). It showed that PGE2 upregulated the proapoptotic gene expressions of CHOP, Bax, and Bak but downregulated pro-survival genes GRP78 and XBPI. By treatment with TMF for 48 h, the mRNA expressions of CHOP, Bax, Bak, GRP78, and XBPI showed an opposite difference from those in model groups and exhibited chondroprotective activity in a dose-dependent manner.
Figure 2: TMF inhibited chondrocytes apoptosis induced by 1 μM PGE₂. The normal chondrocytes were incubated for 48 h. In the control group (a), chondrocytes were incubated with no medicines. Model group (b) was the normal chondrocytes incubated with 1 μM PGE₂. Figures (c)–(e) were the groups incubated with 1 μM PGE₂ and 5, 10, and 20 μg/mL TMF, respectively. Figure (f) was the summarized data obtained from flow cytometry to indicate the rate of apoptosis cells. Data were expressed by mean ± SD of 4 replicates. *p < 0.05 as compared with control.
used PGE₂ found that the protein expression of GSK-3β group. But the expression of XBP1 was upregulated. We also dose-dependently, as compared with those in the model CHOP, BAX, and BAK were significantly downregulated by TMF. This might indicate that TMF could be a potential inhibitor of ERS to protect chondrocytes. Induction of CHOP is considered as a critical event for ERS-mediated apoptosis. CHOP has been demonstrated to directly contribute to apoptosis induced by cytokines by activating mitochondrial apoptosis pathways in β-cells [17]. In accordance with this, PGE₂ could significantly upregulate the expression of CHOP in mRNA and protein levels, which also indicated activation of ERS in chondrocyte cells model. The UPR can be regulated by the activation of ATF4 and ATF6, which are two transcription factors that could activate the expression of CHOP. Released from GRP78, activated phosphorylated PERK phosphorylates eIF2α, which downregulates the translation via inhibiting the activity of the guanine nucleotide exchange factor eIF2B and blocking of eIF2-GDP recycled to eIF2-GTP [18]. However, ATF4 is activated to induce stress responsive genes, such as proapoptotic CHOP [19]. Similar to PERK, active ATF6 is translocated to the Golgi to be cleaved. The cleavage is then translocated to the nucleus to promote the stress responsive genes expression [20]. By treatment with TMF, the expressions of PERK, p-PERK, eIF2α, p-eIF2α, and ATF6 were significantly downregulated.

On the other hand, GRP78, an endoplasmic reticulum chaperone, plays an important protective role in protein processing and provision in UPR [21]. IRE1α possesses both kinase and ribonuclease activity. IRE1α is inactivated by binding to GRP78. Activation of ERS promotes the release of IRE1 from GRP78, leading to oligomerization, autophosphorylation, and activation of IRE1. The mRNA of XBP1 is spliced by the active IRE1a to produce and accumulate the active XBP1, which upregulates the expression of chaperones, such as GRP78 [20]. Active XBP1 exerts its prosurvival effect under conditions of ERS through interacting with Hsp72, which is reported to inhibit the activities of CHOP and JNK [22]. Our findings revealed that the mRNA expressions of GRP78 and XBP1 were significantly upregulated, after treatment for 48 h with TMF. IRE1α activation has been reported to interact with tumor necrosis factor receptor-associated factor 2 (TRAF2) to promote a cascade of phosphorylation events, including activation of the MAP3K apoptosis signal-regulated kinase 1 (ASK1) and JNK [23], which is an important factor in procaspase-9 and Bim can be phosphorylated by JNK to exhibit their proapoptotic roles involved in the activation of BAX and BAK, which are found to directly interact with IRE1a to form a protein complex and to regulate its activity [24]. Stimulated by PGE₂, the expressions of JNK, BAX, and BAK were significantly upregulated but decreased with treatment with TMF for 48 h.

4. Discussion

Apoptosis has been implicated in the progression of chronic degenerative conditions, such as osteoarthritis. Chondrocytes, the unique cell type in cartilage, are implicated in a broad range of substrates and is involved in cell activation, differentiation, and survival [25]. It has been demonstrated that GSK-3β plays an important positive role in the inflammatory process [26]. Also, GSK-3β has been associated with ERS-induced apoptosis. Studies show that GSK-3β inhibitors
reduce the apoptosis induced by ERS, and valproate (2-propylpentanoic acid), via inhibiting the activity of GSK-3β, inhibits ERS-induced apoptosis [27]. By treatment with GSK-3β siRNA, the apoptosis induced by ERS stimulants thapsigargin (TG) and TM can be abrogated [28]. Similarly, in PC12 cells and HepG2 cell, GSK-3β inhibitors can also significantly attenuate TG-induced apoptosis [29]. Our findings showed that the inflammatory cytokine PGE2-induced upregulation of GSK-3β might be associated with progression of ERS and chondrocytes apoptosis, which should be further investigated. These would be inhibited by administration of TMF, which is rich in Chinese traditional medicine and fruits and became a potential drug for chondrocytes protection.

In summary, the inflammatory cytokine PGE2 can activate ERS signaling and promote chondrocytes apoptosis, which might be associated with upregulation of GSK-3β.

TMF exhibits a chondroprotective role in inhibiting PGE2-induced ERS and GSK-3β.

**Conflict of Interests**

The authors declare that they have no conflict of interests.

**Authors’ Contribution**

Jianqiong Yang, Haiqing Liu, and Linfu Li contributed equally to this study.

**Acknowledgments**

This study was financially supported by the National Science Foundation of China (81360277 and 31460082) and the National Science Foundation of Jiangxi Province (20142BAB215047 and 20142BAB215069).
References

[1] K. Takada, J. Hirose, K. Senba et al., “Enhanced apoptotic and reduced protective response in chondrocytes following endoplasmic reticulum stress in osteoarthritic cartilage,” International Journal of Experimental Pathology, vol. 92, no. 4, pp. 232–242, 2011.

[2] R. P. Boot-Handford and M. D. Briggs, “The unfolded protein response and its relevance to connective tissue diseases,” Cell and Tissue Research, vol. 339, no. 1, pp. 197–211, 2010.

[3] H. Nishioto, “CHOP is a multifunctional transcription factor in the ER stress response,” Journal of Biochemistry, vol. 151, no. 3, pp. 217–219, 2012.

[4] A. E. Nugent, D. M. Speicher, I. Gradisar et al., “Advanced osteoarthritis in humans is associated with altered collagen VI expression and upregulation of ER-stress markers Grp78 and Bag-1,” Journal of Histochemistry and Cytochemistry, vol. 57, no. 10, pp. 923–931, 2009.

[5] K. A. Piróg, A. Irman, S. Young et al., “Abnormal chondrocyte apoptosis in the cartilage growth plate is influenced by genetic background and deletion of CHOP in a targeted mouse model of pseudoachondroplasia,” PLoS ONE, vol. 9, no. 2, Article ID e85145, 2014.

[6] Y. Li, Y. Guo, J. Tang et al., “New insights into the roles of CHOP-induced apoptosis in ER stress,” Acta Biochimica et Biophysica Sinica, vol. 46, no. 8, pp. 629–640, 2014.

[7] S. Kim, Y. Joe, H. J. Kim et al., “Endoplasmic reticulum stress-induced IRE1α activation mediates cross-talk of GSK-3β and XBP-1 to regulate inflammatory cytokine production,” The Journal of Immunology, vol. 194, no. 9, pp. 4498–4506, 2015.

[8] D. Liu, H. Zhang, W. Gu, Y. Liu, and M. Zhang, “Ginsenoside Rb1 protects hippocampal neurons from high glucose-induced neurotoxicity by inhibiting GSK3β-mediated CHOP induction,” Molecular Medicine Reports, vol. 9, no. 4, pp. 1434–1438, 2014.

[9] L. Wu, H. Liu, L. Li et al., “5,7,3′,4′-Tetramethoxyflavone exhibits chondroprotective activity by targeting β-catenin signaling in vivo and in vitro,” Biochemical and Biophysical Research Communications, vol. 452, no. 3, pp. 682–688, 2014.

[10] J. Averous, A. Bruhat, C. Joussé, V. Carraro, G. Thiel, and P. Fafournoux, “Induction of CHOP expression by amino acid limitation requires both ATF4 expression and ATF4 phosphorylation,” Journal of Biological Chemistry, vol. 279, no. 7, pp. 5288–5297, 2004.

[11] S.-A. Yoo, S. You, H.-J. Yoon et al., “A novel pathogenic role of the ER chaperone GRP78/BiP in rheumatoid arthritis,” The Journal of Experimental Medicine, vol. 209, no. 4, pp. 871–886, 2012.

[12] H. Sha, Y. He, H. Chen et al., “The IRE1alpha-XBP1 pathway of the unfolded protein response is required for adipogenesis,” Cell Metabolism, vol. 6, pp. 556–564, 2009.

[13] S. F. Wen, V. Mahavni, E. Quijano et al., “Assessment of p53 gene transfer and biological activities in a clinical study of adenovirus-p53 gene therapy for recurrent ovarian cancer,” Cancer Gene Therapy, vol. 10, no. 3, pp. 224–238, 2003.

[14] T. D. Ainsworth, K. Wasmund, L. Ukani et al., “Defining the tipping point: a complex cellular life/death balance in corals in response to stress,” Scientific Reports, vol. 1, article 160, 2011.

[15] Y.-C. Lu, J. Song, H.-Y. Cho, G. Fan, K. K. Yokoyama, and R. Chiu, “Cyclophilin a protects Peg3 from hypermethylation and inactive histone modification,” The Journal of Biological Chemistry, vol. 281, no. 51, pp. 39081–39087, 2006.

[16] Z. Rasheed and T. M. Haqqi, “Endoplasmic reticulum stress induces the expression of COX-2 through activation of eIF2α, p38-MAPK and NF-κB in advanced glycation end products stimulated human chondrocytes,” Biochimica et Biophysica Acta, vol. 1823, no. 12, pp. 2179–2189, 2012.

[17] F. Allagnat, M. Fukaya, T. C. Nogueira et al., “C/EBP homologous protein contributes to cytokine-induced pro-inflammatory responses and apoptosis in β-cells,” Cell Death and Differentiation, vol. 19, no. 11, pp. 1836–1846, 2012.

[18] R. C. Wek, H.-Y. Jiang, and T. G. Anthony, “Coping with stress: eIF2 kinases and translational control,” Biochemical Society Transactions, vol. 34, no. 1, pp. 7–11, 2006.

[19] H. P. Harding, Y. Zhang, H. Zeng et al., “An integrated stress response regulates amino acid metabolism and resistance to oxidative stress,” Molecular Cell, vol. 11, no. 3, pp. 619–633, 2003.

[20] H. Yoshida, T. Okada, K. Haze et al., “ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response,” Molecular and Cellular Biology, vol. 20, no. 18, pp. 6755–6767, 2000.

[21] K. Kogure, K. Nakamura, S. Ikeda et al., “Glucose-regulated protein, 78-kilodalton is a modulator of luteinizing hormone receptor expression in luteinizing granulosa cells in rats,” Biology of Reproduction, vol. 88, no. 1, article 8, 2013.

[22] S. Gupta, A. Deepti, S. Deegan, F. Lisbona, C. Hetz, and A. Samali, “HSP72 protects cells from ER stress-induced apoptosis via enhancement of IRE1α-XBP1 signaling through a physical interaction,” PLoS Biology, vol. 8, no. 7, Article ID e1000410, 2010.

[23] R. Jäger, M. J. M. Bertrand, A. M. Gorman, P. Vandenabeele, and A. Samali, “The unfolded protein response at the crossroads of cellular life and death during endoplasmic reticulum stress,” Biology of the Cell, vol. 104, no. 5, pp. 259–270, 2012.

[24] U. Woehlbi er and C. Hetz, “Modulating stress responses by the UPRosome: a matter of life and death,” Trends in Biochemical Sciences, vol. 36, no. 6, pp. 329–337, 2011.

[25] P. Cohen and M. Goedert, “GSK3 inhibitors: development and therapeutic potential,” Nature Reviews Drug Discovery, vol. 3, no. 6, pp. 479–487, 2004.

[26] E. Beurel, S. M. Michalek, and R. S. Jope, “Innate and adaptive immune responses regulated by glycogen synthase kinase-3 (GSK3),” Trends in Immunology, vol. 31, no. 1, pp. 24–31, 2010.

[27] A. J. Kim, Y. Shi, R. C. Austin, and G. H. Wernstuck, “Valproate protects cells from ER stress-induced lipid accumulation and apoptosis by inhibiting glycogen synthase kinase-3,” Journal of Cell Science, vol. 118, no. 1, pp. 89–99, 2005.

[28] S. Srinivasan, M. Oh sugi, Z. Liu, S. Fat rai, E. Bernal-Mizrachi, and M. A. Permutt, “Endoplasmic reticulum stress-induced apoptosis is partly mediated by reduced insulin signaling through phosphatidylinositol 3-kinase/Akt and increased glycogen synthase kinase-3β in mouse insulinoma cells,” Diabetes, vol. 54, no. 4, pp. 968–975, 2005.

[29] T. Takadera, R. Yoshikawa, and T. Ohyashiki, “Thapsigargin-induced apoptosis was prevented by glycogen synthase kinase-3 inhibitors in PC12 cells,” Neuroscience Letters, vol. 408, no. 2, pp. 124–128, 2006.