Abstract. Venous endothelial cell apoptosis can be induced by endoplasmic reticulum (ER) stress, thus serving an important role in the formation of deep venous thrombosis. X-box binding protein 1 (XBP1) is a protein associated with ER. The present study aimed to explore the function of XBP1/C/EBP homologous protein (CHOP) pathway in the process of endothelial cell apoptosis under hyperglycemia. Small interfering (si)RNAs targeting XBP1 and CHOP were designed to downregulate the expression of XBP1 and CHOP in human umbilical vein endothelial cell, respectively. Flow cytometry was used to determine cell apoptosis. The expression of XBP1, glucose-regulated protein 78 (GRP78), CHOP, Puma, cleaved caspase-3 and Cytochrome c was evaluated by western blotting. There were seven groups of cells that were used in the present study: i) Control (5.5 mM D-glucose); ii) hypertonic (hypertonic control, 27.8 mM mannitol and 5.5 mM D-glucose); iii) 16.7 mM D-glucose; iv) 33.3 mM D-glucose; v) 33.3 mM + NC (33.3 mM D-glucose incubated with NC); vi) 33.3 mM + si-XBP1 (33.3 mM D-glucose incubated with siRNA against XBP1); and vii) 33.3 mM + si-CHOP (33.3 mM D-glucose incubated with siRNA against CHOP). Compared with the control, the apoptosis rate of human umbilical vein endothelial cells (HUVECs) increased greatly with the increase in the concentration of D-glucose. Compared with the 33.3 mM D-glucose group, the HUVECs incubated with 33.3 mM D-glucose and si-XBP1 or 33.3 mM D-glucose and si-CHOP demonstrated a significantly lower apoptosis rate. Compared with the control, XBP1, GRP78, CHOP, Puma, cleaved caspase-3 and cytochrome c were significantly upregulated in the hypertonic, 16.7 mM D-glucose, 33.3 mM D-glucose and 33.3 mM + negative control (NC) groups. Compared with the 33.3 mM D-glucose group, the expression levels of XBP1, GRP78, CHOP, Puma, cleaved caspase-3 and cytochrome c in the 33.3 mM + si-XBP1 or 33.3 mM + si-CHOP groups significantly decreased. High dosage of glucose induced endothelial cell apoptosis by promoting the expression of apoptotic proteins by activating endoplasmic reticulum stress. XBP1/CHOP may be a potential target for the treatment of deep vein thrombosis as one of the key pathways regulating ERS by regulating apoptosis of endothelial cells.

Introduction

Deep venous thrombosis (DVT) is a major threat to psychological health (1). It has high morbidity and its early diagnosis is difficult, as evidence by ~100,000 patients being diagnosed with DVT between 2007-2016 in China alone (2). The pathogenesis of DVT is complicated, in which endothelial cells, leukocytes, platelets, coagulation factors and the fibrinolytic system are involved (3). The structural disorder and dysfunction of venous endothelial cells are the initiating factors of DVT, which impact the development and process of DVT by regulating the systole and diastole of vessels (4). Structural disorder and dysfunction of venous endothelial cells modulates the adherence, activation, recruitment and interaction between platelets and leukocytes and disrupts the balance of coagulation/anticoagulation and fibrinolyis/antifibrinolysis (4). The endothelial cell injury caused by endoplasmic reticulum stress (ERS) serves an important role in the formation of DVT (5). Endothelial cells protect the vessels as the first barrier by regulating blood flow, participating in material exchange, preventing lipid leakage and inhibiting platelet aggregation and thrombogenesis (6). In addition, endothelial cells, especially in new vessels, exert a secretory function as the highly-developed endoplasmic reticulum is visible under an electron microscope, which makes endothelial cells highly allergic to the factors that induce ERS, including Ca2+ metabolism imbalance and oxidative stress stimulation (7,8). ERS is reported to be involved in multiple types of vascular diseases, such as atherosclerosis (9) and Kawasaki disease (10) by inducing the apoptosis of endothelial cells (11). Transcriptional factor X-box binding protein 1 (XBP1) is an important mediator.
in the process of ERS signal transduction in mammalian cells (12). With the development of ERS, inositol-requiring kinase1 (IRE1), an important transmembrane protein molecule in the endoplasmic reticulum cavity, disconnects with glucose-regulated protein 78 to be oligomerized and auto-phosphorylated, hence inducing specific splicing on XBP1 mRNA to combine with ERS reaction components in the nucleus, such as the unfolded protein reaction target molecule glucose-regulated protein 78 (GRP78) (13,14). As a result, the relative expression level of certain ERS-related genes, including activating transcription factor 6 (ATF6) and eukaryotic translation initiation factor 2 (eIF2a) (15), is elevated at the transcriptional level (16).

XBP1 is a novel protein that is closely related to protein folding and endoplasmic reticulum construction; it is an important transcriptional factor in the leucine zipper protein family (17). XBP1 functions as a significant signal regulator for the ERS reaction by binding with the X box cis-acting element located in the promoter region of the major histocompatibility complex gene (18). In the process of ERS, the unspliced X-box binding protein-1 (XBP1-u) composed of 261 amino acids is transformed to spliced X-box binding protein-1 (XBP1-s) composed of 376 amino acids via transcriptional activation in the presence of IRE1. XBP1-s-regulated ERS normally promotes the survival of cells at the early stage of diseases (19,20). However, the persistent activation of ERS will finally result in tissue over-apoptosis with the continuous activation of ERS (21). In addition, C/EBP homologous protein (CHOP) is one of the important factors involved in the ERS-mediated apoptotic pathway (22). Following ERS, the expression level of CHOP is elevated, which further induces apoptosis (23).

The present study explored the impact of XBP1/CHOP signaling pathway on the apoptosis of endothelial cells under the stimulation of hyperglycemia to provide the fundamental basis for the treatment of DVT. Small interfering (si) RNA technology was used to downregulate XBP1 in HUVECs, followed by stimulation of hyperglycemia and measurement of the change of apoptotic rate and the expression level of downstream proteins of XBP1/CHOP pathway. In addition, CHOP was also knocked down using siRNA, followed by stimulation of hyperglycemia and the change in apoptosis rate was measured. The results of the present study may elucidate a potential biomarker for the clinical diagnosis and treatment of DVT.

Materials and methods

Cell culture and treatments. HUVECs (cat. no. iCell-h110) were purchased from iCell Bioscience Inc. and cultured in DMEM (cat. no. KGM12800S-500; Nanjing KeyGen Biotech Co., Ltd.) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and 100 U/ml penicillin/streptomycin at 37°C with 5% CO2, D-glucose (cat. no. G116307) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. and had a purity >99.5%.

Cell transfection. HUVECs culture medium was changed to DMEM without serum when the density of cells reached 70%. A total of ~125 µl of Opti-MEM (Takara Bio Inc.) was added into 2 Eppendorf tubes with the cells at a density of 1x10^6 cells/tube. One tube was filled with 5 µl of Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) and one was filled with 12.5 µl of siRNA (Takara Bio Inc.). After incubation for 15 min and mixing the 2 tubes, the mixture was added into the 6-well-plate and placed into a cell incubator after the cell density reached 70%. After 4 h of transfection at 37°C, 1 ml of complete DMEM containing 20% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) was added into each well. The sequences of the siRNAs targeting XBP1 and CHOP are shown in Table I. The negative control (NC) for siXBP1 and for siCHOP was non-targeting (Takara Bio, Inc.). The sequences of the NC are presented in Table I. The aforementioned agents were added to the six-well-plate after transfection for 48 h. The cells were collected for subsequent experimentation after another 48 h of incubation at 37°C. Untransfected HUVECs were taken as the control group.

Groups of cells. The use of D-glucose to injure endothelial cells has been previously reported (24), and 5.5 mm is the concentration of D-glucose. The effect of different concentrations of D-glucose on endothelial cell proliferation was assessed by conducting a CCK8 assay (25). It was determined that 33.3 mM D-glucose had the most significant inhibitory effect on cell proliferation (data not shown). There were 7 groups of cells in the present study: i) Control (5.5 mM D-glucose); ii) hypertonic (hypertonic control, 27.8 mM mannitol and 5.5 mM D-glucose); iii) 16.7 mM D-glucose; iv) 33.3 mM D-glucose; v) 33.3 mM + NC (33.3 mM D-glucose incubated with NC); vi) 33.3 mM + si-XBP1 (33.3 mM D-glucose incubated with siRNA against XBP1); and vii) 33.3 mM + si-CHOP (33.3 mM D-glucose incubated with siRNA against CHOP). The 33.3 mM D-glucose group was representative of the hyperglycemic condition.

Western blotting. RIPA lysis buffer (cat. no. P0013D; Beyotime Institute of Biotechnology) was used to isolate the proteins from HUVECs at a density of 1x10^6 cells/group. Protein (~35 µg/lane) was separated on a 12% SDS-polyacrylamide gel. The gel was transferred to a polyvinylidene difluoride membrane (MilliporeSigma). The membrane was blocked with 5% skimmed milk in Tris buffered saline/0.1% Tween-20 (pH 7.4) for 1 h at room temperature and incubated overnight at 4°C with the following primary rabbit anti-human antibodies: XBP1 (1:1,000; cat. no. AF5110; Affinity Biosciences, Ltd.), CHOP (1:1,000; cat. no. AF5110, Affinity Biosciences, Ltd.), GRP78 (1:1,000; cat. no. AF5366; Affinity Biosciences, Ltd.), Puma, (1:1,000; cat. no. AF5173; Affinity Biosciences, Ltd.), caspase-3 (1:1,000; cat. no. Ab2302; Abcam), cytochrome C (1:1,000; cat. no. AF0146; Affinity Biosciences, Ltd.) and GAPDH (1:2,000; cat. no. TA-08; OriGene Technologies, Inc.). A horseradish peroxidase (HRP)-conjugated antibody against rabbit IgG (1:5,000; cat. no. ZB-2305; OriGene Technologies, Inc.) was used as a secondary antibody that was incubated at room temperature for 1.5 h. Blots were incubated with the ECL reagents (Beyotime Institute of Biotechnology) and exposed to Tanon 5200-multi (Tanon Science and Technology Co., Ltd.) to detect protein expression. Image J software V1.8.0 (National Institutes of Health)
was used to quantify the relative expression level of target proteins. GAPDH was used as a loading control. A total of 3 independent experiments were performed.

**Flow cytometry for cell apoptosis.** HUVECs were collected in 1.5 ml tubes. Each tube was added with 10 µl fluorescently labeled Annexin V-FITC reagent [cat. no. AP101-100-kit; Multi Sciences (Lianke) Biotech Co., Ltd.] and 5 µl of propidium iodide (PI) reagent [AP101-100-kit; Multi Sciences (Lianke) Biotech Co., Ltd.]. Each tube was incubated for 10 min at room temperature. Cells (~200 µl) were added into flow tubes containing 2 ml of PBS and tested by flow cytometry (FACSAria III; BD Biosciences). The data were analyzed using FlowJo V10.8 software (BD Biosciences). A total of 3 independent experiments were performed and both early apoptosis and late apoptosis were detected.

**Statistical analysis.** All tests were performed using GraphPad Prism 5 software (GraphPad Inc.) and data were presented as the mean ± SD. Statistically significant differences for continuous variables were determined by one-way ANOVA with the post hoc Tukey’s test. P<0.05 was considered to indicate a statistically significant difference. Three statistical replicates were performed for each experiment.

### Results

**CHOP and XBP1 knockdown in HUVECs.** Western blotting was performed to detect the interference efficiency of CHOP, XBP1 and interference vectors in endothelial cells. Compared with the control (untransfected HUVECs), CHOP was significantly downregulated in the siRNA CHOP-2 and siRNA CHOP-3 groups, especially in the siRNA CHOP-3 group (P<0.05; Fig. 1A). The expression of XBP1 deceased greatly in the siRNA XBP1-3 groups compared with that in the control (P<0.05; Fig. 1B). Hence, siRNA CHOP-3 and siRNA XBP1-3 were selected for use in subsequent experiments.

**Knockdown of CHOP or XBP1 inhibits the apoptosis of HUVECs.** Apoptosis was detected by performing flow cytometry. Compared with the control, the apoptosis rate of HUVECs increased greatly with the increase in the concentration of D-glucose (P<0.05; Fig. 2). Compared with the 33.3 mM D-glucose group, the HUVECs incubated with 33.3 mM D-glucose and si-XBP1 or 33.3 mM D-glucose and si-CHOP showed significantly lower apoptosis rates (P<0.05; Fig. 2). In addition, compared to the 33.3 mM+si-XBP1 group, a slightly lower apoptotic rate was observed in the 33.3 mM+si-CHOP group (Fig. 2). The results revealed that XBP1 and CHOP knockdown suppressed high glucose-induced endothelial cell apoptosis.

**Knockdown of CHOP or XBP1 suppresses the expression of GRP78, Puma, cleaved caspase-3 and cytochrome c.** Western blotting was performed to detect the expression levels of XBP1, GRP78, CHOP, Puma, cleaved caspase-3 and cytochrome C. Compared with the control, XBP1, GRP78, CHOP, Puma, cleaved caspase-3 and cytochrome c were significantly upregulated in the hypertonic, 16.7 mM D-glucose, 33.3 mM D-glucose and 33.3 mM + NC groups (P<0.05; Fig. 3). Compared with the 33.3 mM D-glucose group, the expression levels of XBP1, GRP78, CHOP, Puma, cleaved caspase-3, and cytochrome c in the 33.3 mM+si-XBP1 or 33.3 mM+si-CHOP groups significantly decreased (P<0.05; Fig. 3). In addition, compared with the 33.3 mM + NC group, lowest expression level of Puma was observed in the 33.3 mM+si-XBP1 group, while the lowest expression level of GRP78 and cytochrome c was observed in the 33.3 mM + si-CHOP group (P<0.05; Fig. 3). The results indicated that XBP1 and CHOP knockdown inhibited ER stress and apoptotic gene expression in high glucose treated endothelial cells.

**Table I. Sequences of siRNAs targeting XBP1 and CHOP.**

| siRNAs          | siRNA sequences (5’-3’)               |
|-----------------|-------------------------------------|
| CHOP (human) siRNA-1 | S: AGAAAGAACAGGAGAGGAUGATT A: UCAUUCUCCUGUUCUUUCUTT |
| CHOP (human) siRNA-2 | S: GGAGGAAGACCAAGGGAGAGTT A: UCCUCCUGUCUUUCUCCCTT |
| CHOP (human) siRNA-3 | S: AGGAGAAGGAACAGGAGAATTT A: UUCUCUGCUUCUUUCUCCUTT |
| XBP1 (human) siRNA-1 | S: CGAAGAAGGGCUCGAAUGATT A: UCAUUCGAGCCUUCUUCUGTT |
| XBP1 (human) siRNA-2 | S: AGUGGUAGAUUUAGAGGAATT A: UUCUCUAACACUGACACUTT |
| XBP1 (human) siRNA-3 | S: GGUAAUGACUUCAGAATTT A: AAUCUGAAGAGCUAACUTT |
| NC              | S: UUCUCGAGACUGUCACGUTT A: ACGUGACACGUUCGGAGAATT |

Si, small interfering; CHOP, C/EBP homologous protein; XBP1, X box binding protein 1; NC, negative control; S, sense; A, antisense.
Figure 1. Identification of transfection efficacy. The protein expression levels of (A) CHOP and (B) XBP1-3 were detected by western blotting. *P<0.05, vs. Control. Si, small interfering; CHOP, C/EBP homologous protein; XBP1, X box binding protein 1; control, untransfected HUVECs.

Figure 2. Effects of knocking down XBP1 or CHOP on apoptotic rate. Flow cytometry was performed to detect the effects of Si-XBP1 and Si-CHOP on the apoptosis of high glucose induced endothelial cells. The gating strategies for the flow cytometry assay were as follows: i) Early stage of HUVECs apoptosis (Annexin V+PI−); ii) advanced stage of HUVECs apoptosis (Annexin V+PI+); iii) normal HUVECs (Annexin V−PI−); and iv) necrotic HUVECs (Annexin V−PI+). *P<0.05 vs. Control, #P<0.05 vs. Hypertonic, @P<0.05 vs. 16.7 mM D-glucose, ^P<0.05 vs. 33.3 mM D-glucose, &P<0.05 vs. 33.3 mM+NC. Si, small interfering; NC, negative control; CHOP, C/EBP homologous protein; XBP1, X box binding protein 1; PI, propidium iodide; control, untransfected and untreated HUVECs.
Discussion

Normal venous endothelium has antithrombosis effects and venous wall injury is one of the important factors that can result in thrombogenesis (26). Local continuous platelet aggregation adheres to the endothelium when collagen is exposed due to endothelial cell injury, meanwhile, the coagulation system is initiated. The permeability of the endothelium is promoted by the dysfunction of the endothelium, which results in the adhesion of leucocytes to release certain inflammatory factors, such as IL-6, TNF-α and IL-1β. Fibrin deposition is inhibited by the released inflammatory factors to suppress the fibrinolytic system, which contributes to the formation of the prethrombotic state (27). High glucose concentration-induced ERS results in the dysfunction of endothelial cells, the inhibition of cell proliferation and cell death, thereby contributing to injury to vessels and various vascular diseases, including diabetic vascular disease (28,29). Autophagy, apoptosis, inflammation and senescence of endothelial cells can be induced by high dosage of glucose (30). XBP1 is a central regulator in the
process of ERS signal transfer in mammals (19). When ERS arises in the cells, IRE1, which is located in the endoplasmic reticulum, is separated from GRP78 to be activated by oligomerization and autoprophyloration, resulting in the specific splicing of XBP1 mRNA. The expression of ERS-related genes is upregulated when XBP1-s binds with the ERS reaction components in the nucleus (16,31,32). The survival rate of cells is promoted by activating IRE1 artificially under ERS, which indicates that XBPI serves an important role in cell survival and apoptosis (33). XBPI is reported to induce endothelial cell injury, cell apoptosis and coagulation leading to thrombogenesis (34). CHOP is an important signal factor mediating ERS and cell apoptosis; it induces cell apoptosis through excessive ER stress (35). ERS was initiated in primary neonatal mouse cardiomyocytes by stimulation with high concentration of glucose and the expression of XBPI and CHOP was observed to be upregulated (36). By inhibition of the activation of XBPI or downregulation of the expression of XBPI, XBPI splicing was suppressed and CHOP was upregulated, which indicated that the transcription and expression of CHOP could be regulated by XBPI to induce the apoptosis of mouse cardiomyocytes (36). Consistent with the above reports, in the present study, elevated expression level of both XBPI and CHOP could be induced by high glucose concentration. ERS in endothelial cells, denoted by upregulated XBPI and CHOP, was stimulated by treatment with 16.7 mM glucose, which induced a 1 and 2-fold increase in the expression of XBPI and CHOP, respectively. In the present study, a 0.92 and 2.20-fold increase in the expression level of XBPI and CHOP, respectively were observed in 33.3 mM glucose treated endothelial cells. In the present study, the findings related to apoptosis demonstrated that it was induced by the upregulation of XBPI and CHOP and high concentrations of glucose in a dose-dependent manner, which indicated that the XBPI/CHOP signaling pathway exerted important roles in the processing of endothelial cell apoptosis. On the contrary, apoptosis was suppressed by downregulating the expression of XBPI or CHOP, which further verified the involvement of XBPI and CHOP in the process of high glucose induced apoptosis.

GRP78 is a type of molecular chaperone in the endoplasmic reticulum, and high expression of GRP78 can be regarded as the symbol of ERS (37). The increased distribution of GRP78 on the cell membrane exerts a regulatory function on cell apoptosis and cell proliferation (38). GRP78 and CHOP were upregulated in Sertoli cells by a high dosage of glucose, which indicated that the activation of the CHOP signaling pathway under ERS is the mechanism underlying the pro-apoptotic effects of high glucose concentration (39). In the present study, it was found that the expression of GRP78 was upregulated by a high dosage of glucose, which was reversed by downregulating the expression level of XBPI or CHOP.

Puma was upregulated under hyperglycemia in Schwann cells and Cazanave et al (46) claimed that the mRNA and protein level of CHOP in adipocytes was positively related to that of Puma. In the present study, the expression of Puma could be elevated under high glucose concentrations. Approximately 1.1 and 1.9-fold increases in Puma expression were observed in 16.7 mM glucose-treated and 33.3 mM glucose-treated endothelial cells, respectively. In contrast, high protein expression of Puma induced by high concentration of glucose was suppressed by downregulating XBPI or CHOP in the present study.

Caspase-3 is one of the most important apoptotic operators in the caspase family and exists in the cytoplasm as an inactivated proenzyme post synthesis (47). Caspase 3 is activated by stimulating apoptotic signals that degrades multiple types of protein substrates, including pro-caspase-3, pro-caspase-6, pro-caspase-9 and DNA-dependent protein kinase (DNA-PK), in the processing of apoptosis (48). The complex composed of caspase-9 and Cytochrome c is one of the inducers of the activation of caspase-3, which is also reported to be involved in the mitochondrial apoptosis pathway (49). Mitochondrial permeability transition pores are opened under the stimulation of active oxygen and ATP, which contribute to the imbalance of the H+ concentration and differing pressure between the inside and outside of the mitochondria (50). Cytochrome c, which is located within the mitochondria is released into the cytoplasm as a result of differing pressure (31). By binding with caspase-9, cell apoptosis can be induced by Cytochrome c (31). Jiang et al (51) reported that the expression level of cleaved-caspase-3 and Cytochrome c in rat cartilage endplate cells was significantly elevated under hyperglycemia. Similarly, in the present study, the expression of Puma, cleaved caspase-3 and Cytochrome c was upregulated by the high dosage of glucose (33.3 mM), which was reversed by knocking down XBPI and CHOP.

To sum up, in the present study the expression level of proapoptotic proteins, such as GRP78, Puma, caspase-3, and cytochrome c, were elevated by hyperglycemia in a dose-dependent manner, which further contributed to the apoptosis of HUVECs. Following inhibition of the XBPI/CHOP signal pathway, the expression level of pro-apoptotic proteins was suppressed, which further inhibited the apoptosis of endothelial cells. However, the current study has limitations. The present study only investigated the effect of the XBPI/CHOP pathway on ER stress in high glucose induced cells, meaning that the effect of ER stress inhibition on the prevention and treatment of DVT was not investigated in vivo. Future studies should therefore focus on in vivo assessment. Taken together, XBPI/CHOP may be a potential target for the treatment of DVT as one of the key pathways regulating ERS processing through mediating cell apoptosis.

Acknowledgements
Not applicable.

Funding
No funding was received.
Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

MT conceived and designed the current study. JL acquired, analyzed and interpreted the data. YZ performed statistical analysis. YZ made substantial contributions to conception and design, drafted the manuscript, and revised it for important intellectual content. All authors have read and approved the final manuscript. MT and YZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Nicholson M, Chan N, Bhagirath V and Ginsberg J: Prevention of venous thromboembolism in 2020 and beyond. J Clin Med 9: 2467, 2020.
2. Zhang Z, Lei J, Shao X, Dong F, Wang J, Wang D, Wu S, Xie W, Wan J, Chen H, et al: Trends in hospitalization and In-Hospital mortality from VTE, 2007 to 2016, in China. Chest 155: 167‑171, 2017 (In Chinese).
3. Zhao N, Zhang J, Jiang T, Chen X, Wang J, Ding C, Liu F, Qian K and Jiang R: Risk factors of deep venous thrombosis associated with peripherally inserted central venous catheter in upper extremity in ICU. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 29: 2467, 2020.
4. Huang Y, Wang G, Jia H and Liu C: Berberine protects Kawasaki disease‑induced human coronary artery endothelial cells. Biomed Pharmacother 93: 788‑795, 2017.
5. Qi AL, Wu Y, Dong N, Chai YF, Zhu XM and Yao YM: Role of endoplasmic reticulum stress signalling in diabetic endothelial dysfunction and atherosclerosis. Diab Vasc Dis Res 14: 14‑23, 2017.
6. Dong Y, Fernandes C, Liu Y, Wu Y, Wu H, Brophy ML, Deng L, Song K, Wen A, Wong S, et al: Role of endoplasmic reticulum stress signalling in diabetic endothelial dysfunction and atherosclerosis. Diab Vasc Dis Res 13: 788‑795, 2017.
7. Dong Y, Fernandes C, Liu Y, Wu Y, Wu H, Brophy ML, Deng L, Song K, Wen A, Wong S, et al: Role of endoplasmic reticulum stress signalling in diabetic endothelial dysfunction and atherosclerosis. Diab Vasc Dis Res 14: 14‑23, 2017.
8. Xu M, Qi Q, Men L, Wang S, Li M, Xiao M, Chen X, Wang S, Wang G, Jia H and Liu C: Berberine protects Kawasaki disease‑induced human coronary artery endothelial cells dysfunction by inhibiting of oxidative and endoplasmic reticulum stress. Vasc Pharmacol 127: 106660, 2020.
9. Xiaxia C, Lei B and Ding Y: Effect of quercetin on vascular endothelial cell injury under glucosamine treatment. Food Sci 34: 224‑228, 2013.
10. Xu M, Ren LP, Wang C, Zhi YJ, Xing HY, Zhao J and Song YG: Role of X-box binding Protein-1 in Fructose-induced de novo Lipogenesis in HepG2 cells. Chin Med J (Engl) 131: 2310‑2319, 2018.
11. Lyu X, Zhang M, Li G, Cai Y, Li G and Qiao Q: Interleukin‑6 production mediated by the IRE1-XBP1 pathway confers radio‑resistance in human papillomavirus-negative oropharyngeal squamous cell carcinoma. Cancer Sci 110: 2471‑2484, 2019.
12. Zang S, Cao M and Fang F: The role of epigallocatechin‑3‑Gallate in autophagy and endoplasmic reticulum Stress (ERS)‑induced apoptosis of human diseases. Med Sci Monit 26: e924558, 2020.
13. Gaballah HH, Zakaria SS, Mwafty SE. Tahooon NM and Ebeid AM: Mechanistic insights into the effects of quercetin and/or GLP‑1 analogue ligarulidine on high‑fat diet/streptozotocin‑induced type 2 diabetes in rats. Biomed Pharmacother 92: 331‑339, 2017.
14. Chen S, Chen J, Hua X, Sun Y, Cui R, Sha J and Zhu X: The emerging role of XBPI in cancer. Biomed Pharmacother 127: 106660, 2020.
15. Jiang M, Yu S, Yu Z, Sheng H, Li Y, Liu S, Warner DS, Paschen W and Yang W: XBPI (X‑Box‑Binding Protein‑1)‑dependent O‑GlcNAcylation is neuroprotective in ischemic stroke in young mice and its impairment in aged mice is rescued by Thiamet‑G. Sci Adv. 4: e18‑04619, 2018.
16. Yoshida H, Oku M, Suzuki M and Mori K: pXBPI(U) encoded in XBPI pre‑mRNA negatively regulates unfolded protein response activator pXBPI(S) in mammalian ER stress response. J Cell Biol 172: 565‑573, 2006.
17. Nekrutenko A and He J: Functionality of unspliced XBPI is required to explain evolution of overlapping reading frames. Trends Genet 22: 645‑648, 2006.
18. Chen L, Zhao M, Li J, Wang Y, Bao Q, Wu S, Deng X, Tang X, Wu W and Liu X: Critical role of X‑box binding protein 1 in NADPH oxidase 4‑triggered cardiac hypertrophy is mediated by receptor interacting protein kinase 1. Cell Cycle 16: 348‑359, 2017.
19. Qi AL, Wu Y, Dong N, Chai YF, Zhu XM and Yao YM: Recombinant human uimusatin improves immune dysfunction of dendritic cells in septic mice by inhibiting endoplasmic reticulum stress‑related apoptosis. Int Immunopharmacol 85: 106643, 2020.
20. Li Y, Guo Y, Tang J, Jiang J and Chen Z: New insights into the roles of CHOP‑induced apoptosis in ER stress. Acta Biochim Biophys Sin (Shanghai) 47: 146‑147, 2015.
21. Qi AL, Wu Y, Dong N, Chai YF, Zhu XM and Miao J: Long noncoding RNA CA7‑4 promotes autophagy and apoptosis via spongingimirR773‑3P and mir5680 in high glucose‑induced vascular endothelial cells. Autophagy 16: 70‑85, 2020.
22. Zhou Y, Qi C, Li S, Shao X and Ni Z: Investigation of the mechanism underlying calcium dobesilate‑mediated improvement of endothelial dysfunction and inflammation caused by high glucose. Mediators Inflamm 2019: 9893682, 2019.
23. Valeriani E, Riva N, Di Nisio M and Ageno W: Splanchnic vein thrombosis: Current perspectives. Vase Health Risk Manag 15: 449‑461, 2019.
24. Mukhopadhyay S, Johnson TA, Duru N, Buzza MS, Pawar NR, Sarkar R and Antalis TM: Fibrinolysis and inflammation in venous thrombus resolution. Front Immunol 10: 1348, 2019.
25. Fan Z, Guo C, Zhang Y, Yao J, Liao L and Dong J: Hongjtingtian injection inhibits proliferation and migration and promotes apoptosis in high glucose‑induced vascular smooth muscle cells. Drug Des Devel Ther 13: 4115‑4126, 2019.
26. Zhang Z, Zhang S, Wang Y, Yang M, Zhang N, Jin Z, Ding L, Jiang W, Yang J, Sun Z, et al: Autophagy inhibits high glucose induced cardiac microvascular endothelial cells apoptosis by mTOR signal pathway. Apoptosis 22: 1510‑1523, 2017.
27. Huang LY, Yan IC, Tsai WC, Ahmetaj‑Shala B, Chang TC, Tsai CS and Lee SY: Rhodiola crenulata attenuates high glucose‑induced endothelial dysfunction in human umbilical vein endothelial cells. Am J Chin Med 45: 1201‑1216, 2017.
28. Chen R, Gao XG and Zhang L: Cannabidiol attenuates palmitic acid‑induced hepatocytes injury through promoting autophagic flux. Acad J Second Military Med Univ 38: 583‑588, 2017.
32. Li LF, Wen Y, Jiang L and Zhu YQ: Establishment of a model of endoplasmic reticulum stress response in dental pulp cells induced by tunicamycin. Shanghai Kou Qiang Yi Xue 27: 135‑138, 2018 (In Chinese).

33. Fink SL, Jayewickreme TR, Moloney RD, Iwawaki T, Landis CS, Lindenbach BD and Iwasaki A: IRE1α promotes viral infection by conferring resistance to apoptosis. Sci Signal 10: eaai7814, 2017.

34. Kelaini S, Caines R, Zeng L and Margariti A: Chapter 13-X‑box‑binding Protein 1 splicing induces an autophagic response in endothelial cells: Molecular mechanisms in ECs and Atherosclerosis. Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging, 259‑268, 2017 doi:10.1016/B978‑0‑12‑805420‑8.00013‑5.

35. Su Q, Wang Y, Yang X, Li XD, Qi YF, He XJ and Wang YJ: Inhibition of endoplasmic reticulum stress apoptosis by estrogen protects human umbilical vein endothelial cells through the PI3 Kinase‑Akt signaling pathway. J Cell Biochem 118: 4568‑4574, 2017.

36. Miyazaki Y, Kaikita K, Endo M, Horio E, Miura M, Tsujita K, Hokimoto S, Yamamuro M, Iwawaki T, Gotoh T, et al: C/EBP homologous protein deficiency attenuates myocardial reperfusion injury by inhibiting myocardial apoptosis and inflammation. Arterioscler Thromb Vasc Biol 31: 1124‑1132, 2011.

37. Staquicini DI, D'Angelo S, Ferrara F, Karjalainen K, Sharma G, Smith TL, Tarleton CA, Jaalouk DE, Kuniyasu A, Baze WB, et al: Therapeutic targeting of membrane‑associated GRP78 in leukemia and lymphoma: Preclinical efficacy in vitro and formal toxicity study of BMTP‑78 in rodents and primates. Pharmacogenomics J 18: 436‑443, 2018.

38. Liang G, Fang X, Yang Y and Song Y: Knockdown of CEMEP suppresses proliferation and induces apoptosis in colorectal cancer cells: Downregulation of GRP78 and attenuation of unfolded protein response. Biochem Cell Biol 96: 332‑341, 2018.

39. Yang Y, Huang H, Feng D, Liu W, Cheng X, Ba Y and Cui L: Effects of N‑acetyl‑l‑tryptophan against hepatic ischemia‑reperfusion injury via the RIP2/caspase‑1/IL‑1β signaling pathway. Pharma Biol 57: 385‑391, 2019.

40. Schubert F, Rapp J, Braun‑Schubert P, Schlicher L, Stock K, Wissler M, Weiß M, Charvet C, Borner C and Maurer U: Requirement of GSK‑3 for PUMA induction upon loss of pro‑survival PI3K signaling. Cell Death Dis 9: 470, 2018.

41. Tiong YL, Ng KY, Koh RY, Ponnudurai G and Chye SM: Melatonin prevents oxidative stress‑induced mitochondrial dysfunction and apoptosis in high glucose‑treated Schwann cells via upregulation of Becl2, NF‑xB, mTOR, Wnt signalling pathways. Antioxidants (Basel) 8: 198, 2019.

42. Liu H, Zhou Y and Tang L: Caffeine induces sustained apoptosis of human gastric cancer cells by activating the caspase9/caspase3 signalling pathway. Mol Med Rep 16: 2445‑2454, 2017.

43. Wang Y, Liu C, Wang J, Zhang Y and Chen L: Iodine‑131 induces apoptosis in human cardiac muscle cells through the p53/Bax/caspase‑3 and PIDD/caspase‑2/t‑BID/cytochrome c/caspase‑3 signaling pathway. Oncol Rep 38: 1579‑1586, 2017.

44. Xie S, Liu J, Zhang Z, Li L and Wei Z: Apoptosis effects of imperatorin on synovioocytes in rheumatoid arthritis through mitochondrial/caspase‑mediated pathways. Food Funct 9: 2070‑2079, 2018.

45. Jiang Z, Lu W, Zeng Q, Li D, Ding L and Wu J: High glucose‑induced excessive reactive oxygen species promote apoptosis through mitochondrial damage in rat cartilage endplate cells. J Orthop Res 36: 2476‑2483, 2018.