J Appl Biomed
DOI: 10.32725/jab.2024.021
journal homepage: http://jab.zsf.jcu.cz

Journal of Applied Biomedicine

Original research article

Syringin protects high glucose-induced BMSC injury, cell senescence, and osteoporosis by inhibiting JAK2/STAT3 signaling

Yu-Cong Zou [1] *, Kai Gao [2], Bao-Tao Cao [2], Xiao-Li He [2], Wei Zheng [2], Xiao-Fei Wang [2], Yu-Fu Li [2], Feng Li [2], Hua-Jun Wang [3] *

[1] *Zhuhai Hospital of Integrated Traditional Chinese & Western Medicine, Department of Rehabilitation, Zhuhai 519020, China*
[2] *Third Hospital of Shijiazhuang, Department of Orthopedic Surgery, HeBei Province 510000, China*
[3] *Jinan University, The First Affiliated Hospital, Department of Bone and Joint Surgery and Sports Medicine Center, Guangzhou, 510630 China*

Abstract
Background: Acanthopanax senticosus (Rupr. et Maxim.) is commonly used in Traditional Chinese Medicine. Syringin is a major ingredient of phenolic glycoside in *Acanthopanax senticosus*.
Objective: This study was performed to investigate whether Syringin could protect high glucose-induced bone marrow mesenchymal stem cells (BMSCs) injury, cell senescence, and osteoporosis by inhibiting JAK2/STAT3 signaling.
Methods: BMSCs isolated from both the tibia and femur of mice were induced for osteogenesis. The cell senescence was induced using the high glucose medium. The cells were treated with 10 and 100 μmol/l Syringin. Immunohistochemistry staining was performed to determine the β-galactosidase (SA-β-gal) levels in differentially treated BMSCs. MTT assay and flow cytometry analysis were also performed to assess cell viability and cell cycle. The level of ROS in cells with different treatment was measured by using flow cytometry with DCF-DA staining. Calcium deposition and mineralized matrices were detected with alizarin red and ALP staining, respectively. Osteogenesis related genes *OCN, ALP, Runx2*, and *BMP-2* were detected by RT-PCR. Levels of senescence-related proteins including p53 and p21, as well as JAK2, p-JAK2, STAT3, and p-STAT3 were detected by Western blot analysis.
Results: Syringin treatment reversed the phenotypes of senescence caused by high glucose in BMSCs, including the arrest of G0/G1 cell cycle, enhanced SA-β-gal activity, and impaired cell growth. Syringin also decreased the elevated ROS production and the levels of p53, p21, and JAK2/STAT3 signaling activation. In addition, Syringin also enhanced the osteogenic potential determined by ARS and ALP staining, as well as increasing *OCN, ALP, Runx2*, and *BMP-2* expressions.
Conclusion: Syringin protects high glucose-induced BMSC injury, cell senescence, and osteoporosis by inhibiting JAK2/STAT3 signaling.

Keywords: BMSC; Cell senescence; Osteoporosis; Syringin; Traditional Chinese Medicine

Highlights:
- Syringin treatment reversed the phenotypes of senescence.
- Syringin enhanced the osteogenic potential.
- Syringin protected high glucose-induced BMSC injury, cell senescence, and osteoporosis by inhibiting JAK2/STAT3 signaling.

Introduction

Osteoporosis is a systemic bone disease – a worldwide metabolic bone disorder with high incidence, identified as decreased bone quality and density. It is caused by the menopause, human ageing, diabetes, and other factors. The World Health Organization (WHO) defined osteoporosis as a "progressive systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture". Undoubtedly, osteoporosis is a growing problem. China spent about $9.45 billion on treating osteoporosis-related fractures in 2010, and it is estimated that this will reach a substantial $25.43 billion by 2050, creating a significant economic burden (Wang et al., 2024).

Caused by diabetes mellitus (DM), diabetic osteoporosis was identified by microarchitectural alterations and low bone mass (Cheng et al., 2024). In comparison to the patients without diabetes, the risk of spinal fracture and hip fracture

* **Corresponding authors:** Yu-Cong Zou, Zhuhai Hospital of Integrated Traditional Chinese & Western Medicine, Department of Rehabilitation, Zhuhai 519020, China; e-mail: zyc0118@smu.edu.cn
Hua-Jun Wang, Jinan University, The First Affiliated Hospital, Department of Bone and Joint Surgery and Sports Medicine Center, Guangzhou 510630, China; e-mail: whjortho@hotmail.com; http://doi.org/10.32725/jab.2024.021

Submitted: 2024-02-06 • Accepted: 2024-10-16 • Prepublished online: 2024-10-21
J Appl Biomed 22/4: 197–207 • EISSN 1214-0287 • ISSN 1214-021X

was significantly increased in the DM patients (2–5 fold and 2–7 fold, respectively) (Shah et al., 2015). DM could suppress the formation and enhance the resorption of bone, subsequently causing bone osteoporosis and bone loss (Martiniakova et al., 2024). DM exerted its inhibitory effect on the formation of bone through inducing the dysfunction of osteoblasts precursors, like the bone marrow mesenchymal stem cells (BMSCs) (Khosla et al., 2021).

Characterized by the loss of proliferative capacity irreversibly and progressively, cellular senescence can be induced by oncogene stimulation, DNA damage, oxidative stress, and telomere shortening (Reimann et al., 2024). In addition to the arrest of the cell growth and changes of functions, senescent cells also produced more senescence-associated secretory phenotype (SASP) factors, such as plasminogen activator inhibitor 1 (PAI1), monocyte chemotactic protein 1 (MCP1), MMP12, matrix metalloproteinase 3 (MMP), CXCL15, chemokine (C-X-C motif) ligand 1 (CXCL1), IL6, and interleukin 1 (IL-1) (Ogrodnik, 2021).

In recent years, high glucose (HG) has been demonstrated to induce cellular senescence of different cell types at different conditions. For example, HG could promote and aggravate the senescence of vascular endothelial cells in women with hyperglycemia in pregnancy (Zheng et al., 2024). Through affecting the signaling of NRSF/REST elevation, high glucose can cause the senescence of neuronal cell (Xue et al., 2022). Moreover, HG also induced senescence of renal tubular cells contributing to diabetic nephropathy (Eleftheriadis et al., 2022). Altered hematopoietic niche and inhibited hematopoietic function were observed in the patients with hyperglycemia (Kojima et al., 2014). Additionally, the expression profiles of chemokines in bone marrow mesenchymal stem cells were changed in the patients with diabetes (Ferraro et al., 2011). The telomere changes, premature senescence, and genomic instability were observed in the bone marrow-derived MSCs after incubation in the medium with HG (Estrada et al., 2013; Luo et al., 2023; Zhai et al., 2020). HG could induce BMSCs senescence by upregulating autophagy (Chang et al., 2015).

Thus, to further prevent the fragility and osteoporosis caused by diabetes, novel therapeutic strategies, or treatments for impairing BMSC senescence are needed urgently.

There are many pharmaceutical treatments for osteoporosis, including hormone-replacement therapy (HRT), bisphosphonates, calcitonin, selective estrogen receptor modulators (SERMs), recombinant human parathyroid hormone (rhPTH), and Vitamin D analogues. Unfortunately, the increase in bone mass with these drugs is very limited (An et al., 2016), and many of them are not recommended for long-term use because of their adverse effects, such as the risk of breast cancer with hormone replacement therapy, or osteonecrosis of the jaw (ONJ) with diphosphates.

Traditional Chinese Medicine (TCM) has been used for more than 8,000 years in China. TCM has undergone some modifications in Japan and Korea, and recently it attracted more attention in North America and Europe. Due to less adverse effects, TCM is a potential candidate for treatment of osteoporosis. In addition, since osteoporosis is a multifactorial disease, Chinese herbal medicine, which consists of natural herbs, has a multi-pathways and multi-targets approach to achieve therapeutic effects, which is more similar to the onset of osteoporosis (Cao et al., 2024). Syringin, also called eleutheroside B, is derived from *Eleutherococcus senticosus* (Araliaceae) (Qian et al., 2024). Previous studies have reported that Syringin possesses broad pharmacological effects, including immunomodulatory, anti-inflammatory, antioxidant, and hepatoprotective (Mao et al., 2023; Singh et al., 2023, 2024). Previously, potent anti-osteoporosis activity was found in ovariectomized mice (Liu et al., 2018). Interestingly, Syringin has also been shown to have an anti-hyperglycemic effect in both *in vivo* and *in vitro* studies (Niu et al., 2007; Shen et al., 2020). However, whether Syringin could inhibit HG induced cell senescence of BMSCs remains unexplored.

Hence, this study was performed to investigate whether Syringin could protect high glucose-induced BMSC injury, cell senescence, and osteoporosis by inhibiting JAK2/STAT3 signaling.

Materials and methods

Cell preparation, high glucose, and Syringin treatment

The BMSCs isolation and culture was conducted according to routine process (Chu et al., 2020). BMSCs were isolated from the femur and tibia of mice, and incubated with FBS (10%) (Gibco), penicillin and streptomycin (1%) (Gibco), and glutamine (Sigma-Aldrich) contained α-MEM medium (Invitrogen). Ascorbic acid (50 μg/ml), β-glycerophosphate (5 mM), and dexamethasone (10 nM) (Sigma-Aldrich) were used to induce the differentiation of osteogenesis. Cells were cultured at 37 °C and the medium was changed every 2–3 days.

To prepare the BMSCs for the HG treatment, after the first passage, the cells were serum starved for 24 hours in LG-DMEM with 5% FBS. Then, the cells were cultured in HG (25 mmol/l) DMEM for two weeks and then harvested for further analysis. Syringin was dissolved in phosphate-buffered saline (PBS) at different concentrations (10 and 100 μmol/l) for dose-dependent treatments. Cells from passages 4 to 6 were used in the experiments.

Measurement of cell viability

After washing with PBS and indicated incubations, the cells (1×10^4 cells/ml in 96-well plate) were incubated with 100 μl 3 MTT solution (Sigma) for 1.5 h. Then, the cell viability was determined at 570 nm after changing the reaction medium with DMSO according to the routine process (Kumar et al., 2018).

Analysis of cell cycle

PI staining was performed to determine the distribution of cell cycle (Pozarowski and Darzynkiewicz, 2004). Briefly, after collection and washing, the BMSCs were incubated with 1 ml DNA staining solution and 10 μl permeabilization solution for 30 min at room temperature under dark conditions. Finally, the distribution of cell cycle was observed and analyzed using flow cytometry (BD Biosciences, San Diego, CA, USA).

Senescence associated β-galactosidase staining

A SA-β-gal staining kit (Cell Signaling Technology) was employed to detect the level of SA-β-gal in BMSCs (Itahana et al., 2007). Briefly, after washing and fixation, the cells were incubated with β-Gal staining solution (final concentration 1 mg/ml of X-gal in DMF) at 37 °C overnight. The results were observed and quantified using a light microscopy.

Quantitative real-time PCR (qPCR) analysis of osteogenesis related gene

After extraction using TRIzol reagent (Invitrogen) and quantification with a NanoDrop ND-1000 Spectrophotometer (Nanodrop Technologies), a high-capacity cDNA reverse transcription kit (Invitrogen) was employed to reverse-transcript the mRNA to cDNA, and qPCR assay was conducted to deter-

mine the levels of candidate genes using the SYBR Green PCR Master mix (Roche) according to the routine process (Ma et al., 2020). The values of gene expression were normalized to the level of GAPDH. The sequences of primers were listed as follows:

OCN: F: 5'-GAGGGCAATAAGGTAGTGAA-3';
R: 5'-CATAGATGCGTTTGTAGGC-3';
Runx2 F: 5'-TTCAACGATCTGAGATTTGTGGG-3';
R: 5'-GGATGAGGAATGCGCCCTA-3';
ALP: F: 5'-GAGCGTCATCCCAGTGGAG-3';
R: 5'-TAGCGGTTACTGTAGACACCC-3';
BMP-2: F: 5'-ATAGCAGTTTCCATCACCGAA-3';
R: 5'-ACTTCCACCACGAAT CCAT-3';
GAPDH: F: 5'-TCACCAGGGCTGCTTTTAAC-3';
R: 5'-GACAAGCTTCCCGTTCTCAG-3'.

Data were analyzed using the $2^{-\Delta\Delta CT}$ method.

Alkaline phosphatase (ALP) and alizarin red staining (ARS)

ALP and ARS staining were carried out to evaluate the differentiation of osteogenesis according to the previously published process (Huang and Wang, 2022). After three-time washing with ddH_2O and one-hour fixation with 70% ethanol, the osteogenically differentiated BMSCs were incubated with Alizarin Red S solution (40 mM) (Sigma-Aldrich) for 15 min. Then the stained cells were observed using a phase contrast microscopy after five-time washing and PBS rinsing.

For ALP staining, after three-time washing and 15-min fixation with 4% PFA, a BCIP/NBTALP Color Development Kit (Beyotime) was employed to stain the cells.

Detection of reactive oxygen species (ROS) level

ROS was detected as previously described (Kou et al., 2023). The BMSCs were stained with DCFH-DA at 37 °C for 20 min after two-time washing with PBS. Then the ROS levels were determined using flow cytometry after washing with serum-free medium.

Western blot analysis

Western blot analysis was performed as previously described (Cai et al., 2022). After washing with PBS and lysed with radio-immunoprecipitation assay buffer, the supernatants were collected by centrifugation at 4 °C, and the protein concentrations were measured. After 10-min boiling with loading buffer, 10 μg protein samples were loaded to 10% SDS-PAGE gel and the protein bands were transferred to a PVDF membrane. After blocking with 5% fat-free milk, the proteins were incubated with indicated primary antibodies: JAK2 (1:1000, Abcam, UK), p-JAK2 (1:1000, Abcam, UK), STAT3 (1:1000, Abcam, UK), p-STAT3 (1:1000, Abcam, UK), p53 (1:2000, Abcam, UK), p21 (1:2000, Abcam, UK), and GAPDH (1:2000, Abcam, UK). After three-time washing with PBST buffer, the proteins were cultured with indicated secondary antibody for 1 h at RT. The signals of the bands were detected, the results analyzed using Image J software (LI-COR Biosciences), and the values quantified and normalized to the expression of GAPDH.

Statistical analysis

All the values in this study are represented as means ± standard deviation (SD). SPSS 21.0 software and GraphPad software were employed to analyze the results and produce figures respectively. The one-way ANOVA was used for statistical analysis. Significant difference was defined as $P < 0.05$. All the results showed in this study represent at least three independent experiments.

Results

Syringin preserves the proliferation potential of BMSCs

The amount of the cells from the groups of control, HG, HG + 10 μmol/l Sy, HG + 100 μmol/l Sy was monitored at day 1, day 3, day 7, and day 14 to evaluate the growth of BMSCs after indicated treatment. As shown in Fig. 1, no differences among these groups of cells were observed during the first week. However, slower proliferation was observed in the HG group of cells compared to the control cells at day 14 (** $P < 0.01$). BMSCs of the groups HG + 10 μmol/l Sy, HG + 100 μmol/l Sy proliferated faster than HG group ($P < 0.05$) (Fig. 1). Thus, Syringin could restore the inhibited cell proliferation of BMSCs caused by HG *in vitro*.

Syringin inhibits the G1 cell cycle arrest of senescent BMSCs

Cell cycle detection showed that the cells in the G1 phase were 62.00 ± 5.37%, 72.64 ± 6.48%, 55.29 ± 4.44%, and 61.30 ± 6.72% for the control groups, HG, HG + 10 μmol/l Sy, and HG + 100 μmol/l Sy, respectively, suggesting the arrest of the cells in G1 phase after HG incubation. However, we observed an obviously reduced G1 arrest and increased number of cells in S phase in the cells treated with HG + 10 μmol/l Sy or HG + 100 μmol/l Sy in comparison to the cells treated with HG only ($P < 0.05$) (Fig. 2A, B). Moreover, there was no significant difference regarding G2 phase among the four groups (Fig. 2A, B). These results indicated that Syringin exerted its protective effect on the senescence of BMSCs caused by HG through impairing the arrest of G1 in the cell cycle.

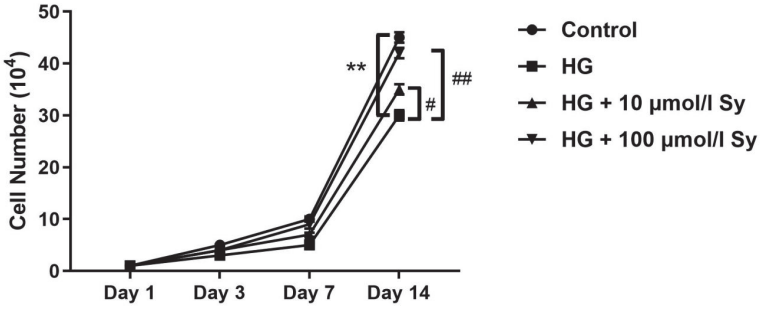

Fig. 1. Growth of BMSCs with differential treatment; control, HG, HG + 10 μmol/l Sy, and HG + 100 μmol/l Sy, respectively. ** $P < 0.01$ compared to the control cells; # and ## represent $P < 0.05$ and $P < 0.01$ compared to HG group of cells, respectively.

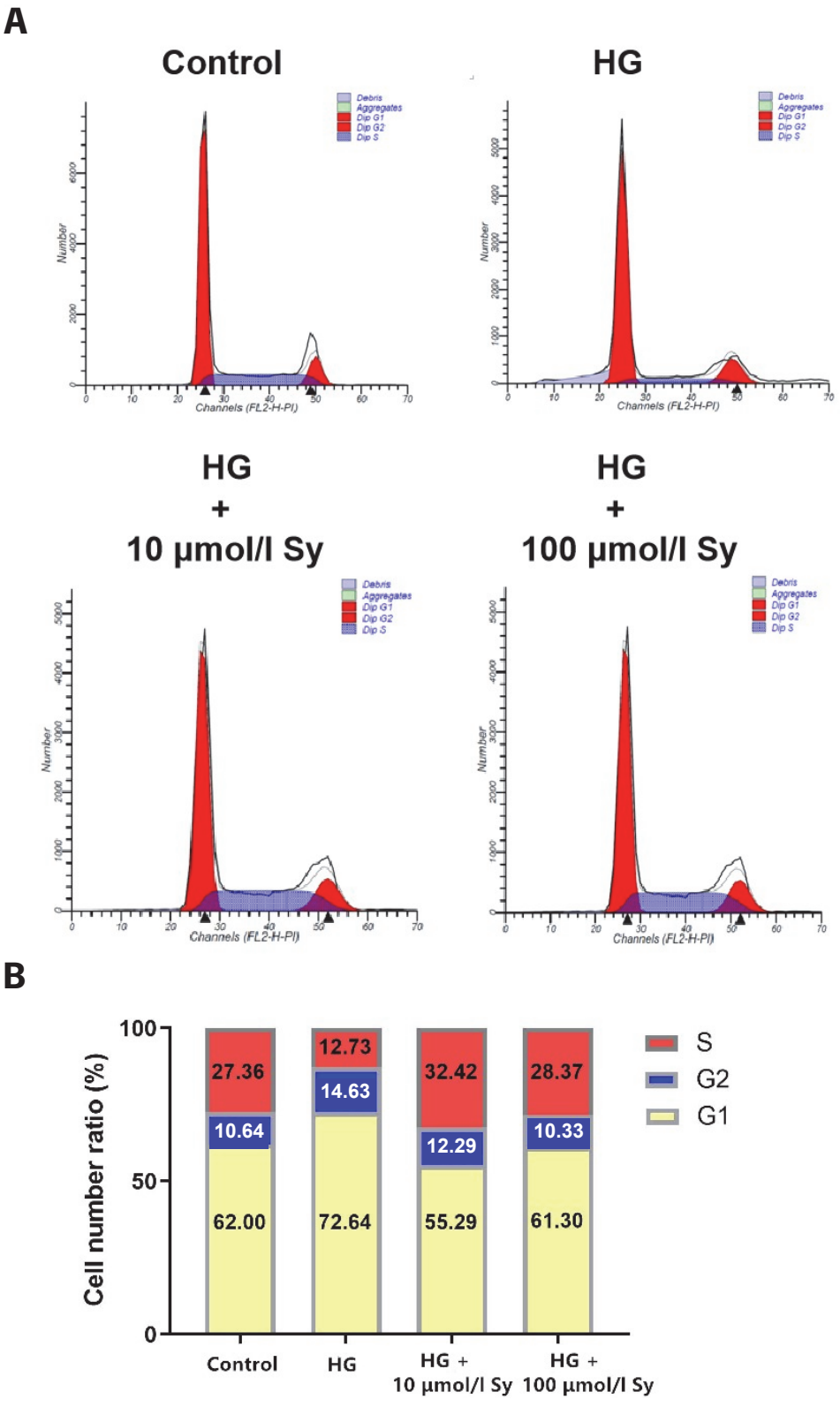

Fig. 2. Cell cycle analysis of BMSCs. (**A**) The cell cycle distributions of the cells from the control, HG, HG + 10 μmol/l Sy, and HG + 100 μmol/l Sy groups were analyzed using flow cytometry. (**B**) Quantitative analysis of cell cycle distribution among the four groups (G1/S: * $P < 0.05$ vs. Control, # $P < 0.05$ vs. HG).

Syringin promotes osteogenic differentiation of BMSCs exposed to high glucose in vitro

The results obtained from ARS and ALP staining showed that, in comparison to cell control group, HG treatment significantly reduced the ALP activity and the formation of mineralized nodule (** $P < 0.01$) (Fig. 3A–C). However, Syringin treatment clearly enhanced the ALP activity and formation of mineralized nodule in the BMSCs treated with HG medium in a dose-dependent manner, suggesting a partial restoration of the osteogenic differentiation which was impaired by HG treatment (Fig. 3A–C) (# $P < 0.05$). Furthermore, the expression of osteo-related genes, such as *BMP2, ALP, OCN*, and *Runx2*, was significantly suppressed by HG treatment compared to the control cells, while this suppressed gene expression caused by HG administration was reversed by Syringin treatment (# $P < 0.05$) (Fig. 4).

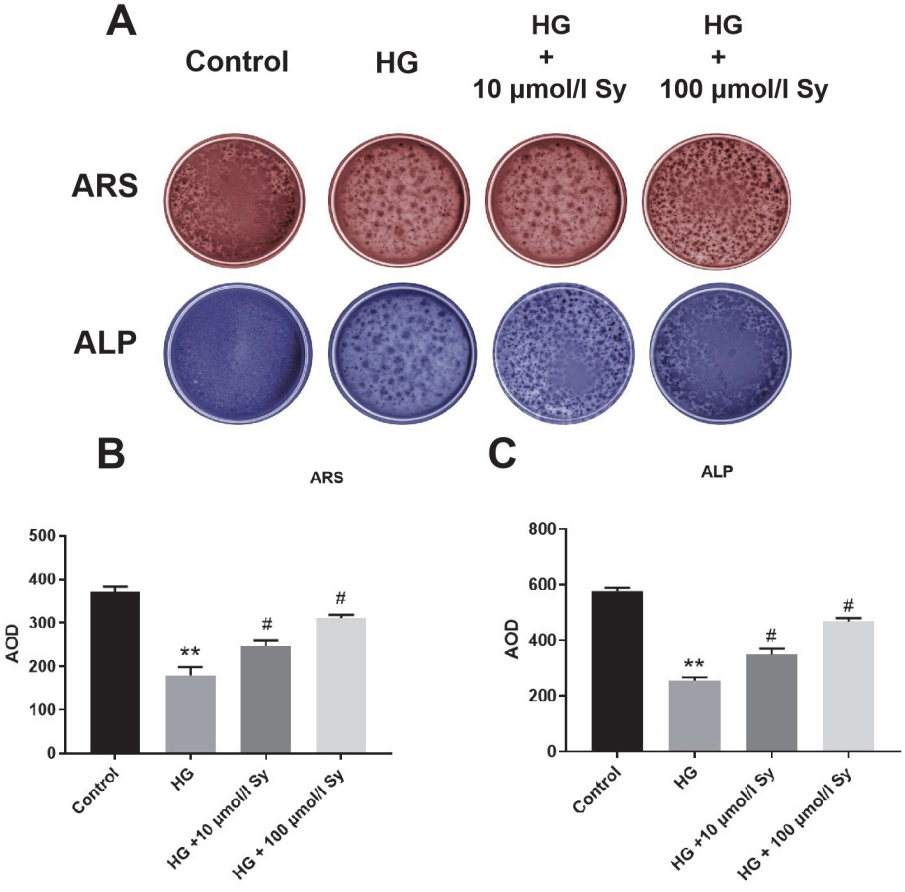

Fig. 3. (**A**) Representative figures of ARS and ALP staining. (**B**) Quantitative analysis of ARS staining among control, HG + 10 μmol/l Sy, and HG + 100 μmol/l Sy. (**C**) Quantitative analysis of ALP staining among control, HG + 10 μmol/l Sy, and HG + 100 μmol/l Sy (** $P < 0.01$ vs. control; # $P < 0.05$ vs. HG).

Syringin reduces SA-β-Gal activity in BMSCs

Our SA-β-Gal staining results showed that the IOA value (integrated option density (IOD)/Area) of the SA-β-Gal-expressed BMSCs were 0.12 ± 0.03, 2.21 ± 0.04, 1.32 ± 0.20 and 0.36 ± 0.01 for the control group, HG group, HG + 10 μmol/l Sy, and HG + 100 μmol/l Sy, respectively (Fig. 5A, B). There was a significantly increased number of SA-β-gal positive BMSCs in the HG group compared to the control group (** $P < 0.01$). However, fewer SA-β-Gal positive BMSCs were observed in the groups of cells treated with HG + 10 μmol/l Sy or HG + 100 μmol/l Sy in comparison to the only HG treated cells ($P < 0.05$) (Fig. 5A, B). These findings indicated that Syringin significantly protected the BMSCs from HG induced senescence.

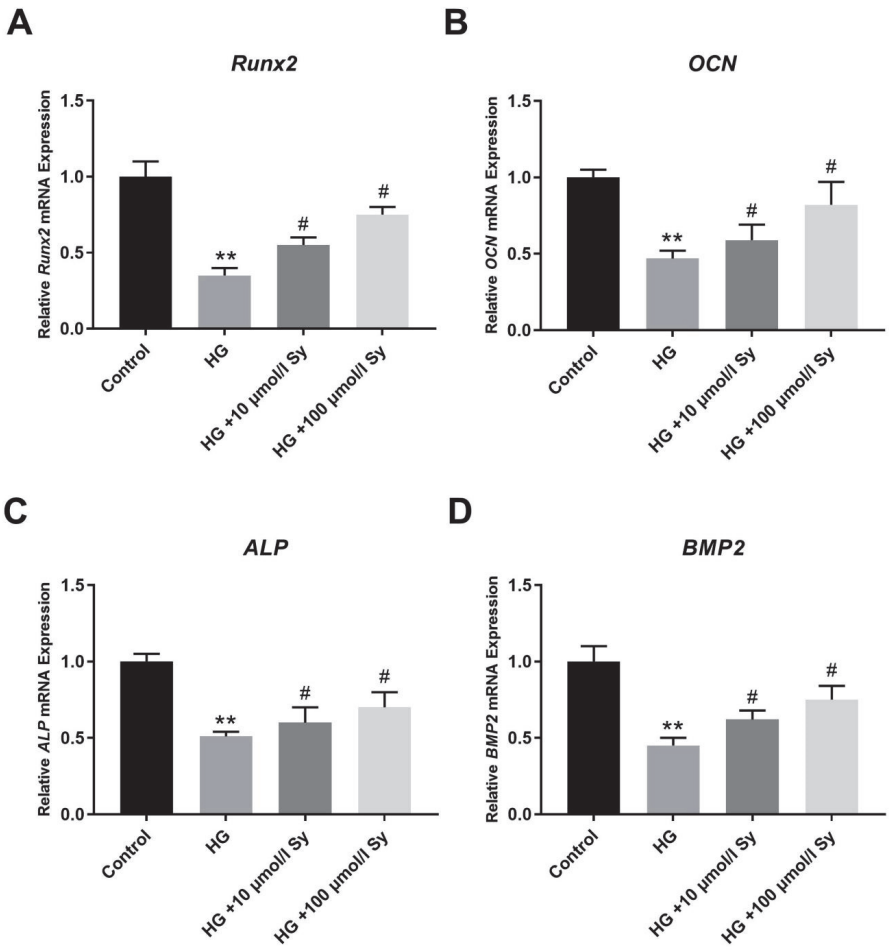

Fig. 4. (**A–D**) Comparison of osteogenesis-related gene Runx2, OCN, ALP, and BMP2 between control, HG, HG + 10 μmol/l Sy, and HG + 100 μmol/l Sy (** $P < 0.01$ vs. control; # $P < 0.05$ vs. HG).

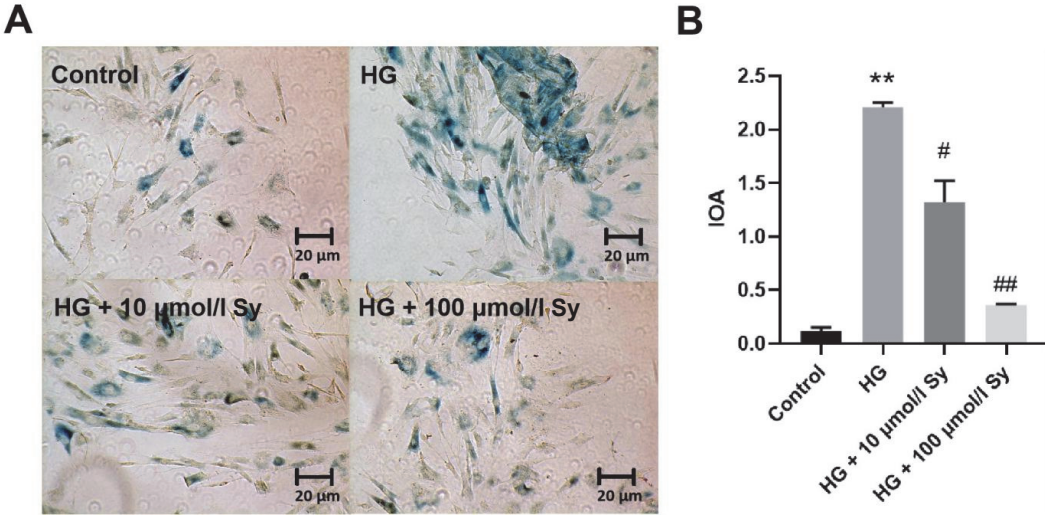

Fig. 5. The detection of SA-β-Gal activity in BMSCs. (**A**) SA-β-Gal positive BMSCs in the control group, HG group, HG + 10 μmol/l Sy, and HG + 100 μmol/l Sy were detected using a SA-β-Gal staining kit. (**B**) The intensity of area of SA-β-Gal positive BMSCs in different groups. Syringin significantly reduced SA-β-Gal activity in BMSCs from the HG + 10 μmol/l Sy and HG + 100 μmol/l Sy in comparison to BMSCs from the HG group. ** $P < 0.01$ vs. control group, # $P < 0.05$ vs. HG group, ## $P < 0.01$ vs. HG group.

Syringin attenuates the levels of ROS

To examine the effect of Syringin on the production of ROS in BMSCs, the levels of ROS in the cells were measured. Compared to the control group of cells, HG group of cells had significantly higher level of ROS (** $P < 0.01$) (Fig. 6A, B), while Syringin at both 10 μmol/l and 100 μmol/l significantly decreased ROS levels in HG induced aging BMSCs (# $P < 0.05$~ ## $P < 0.01$) (Fig. 6A, B), suggesting the inhibition of Syringin on HG-caused ROS generation in BMSCs.

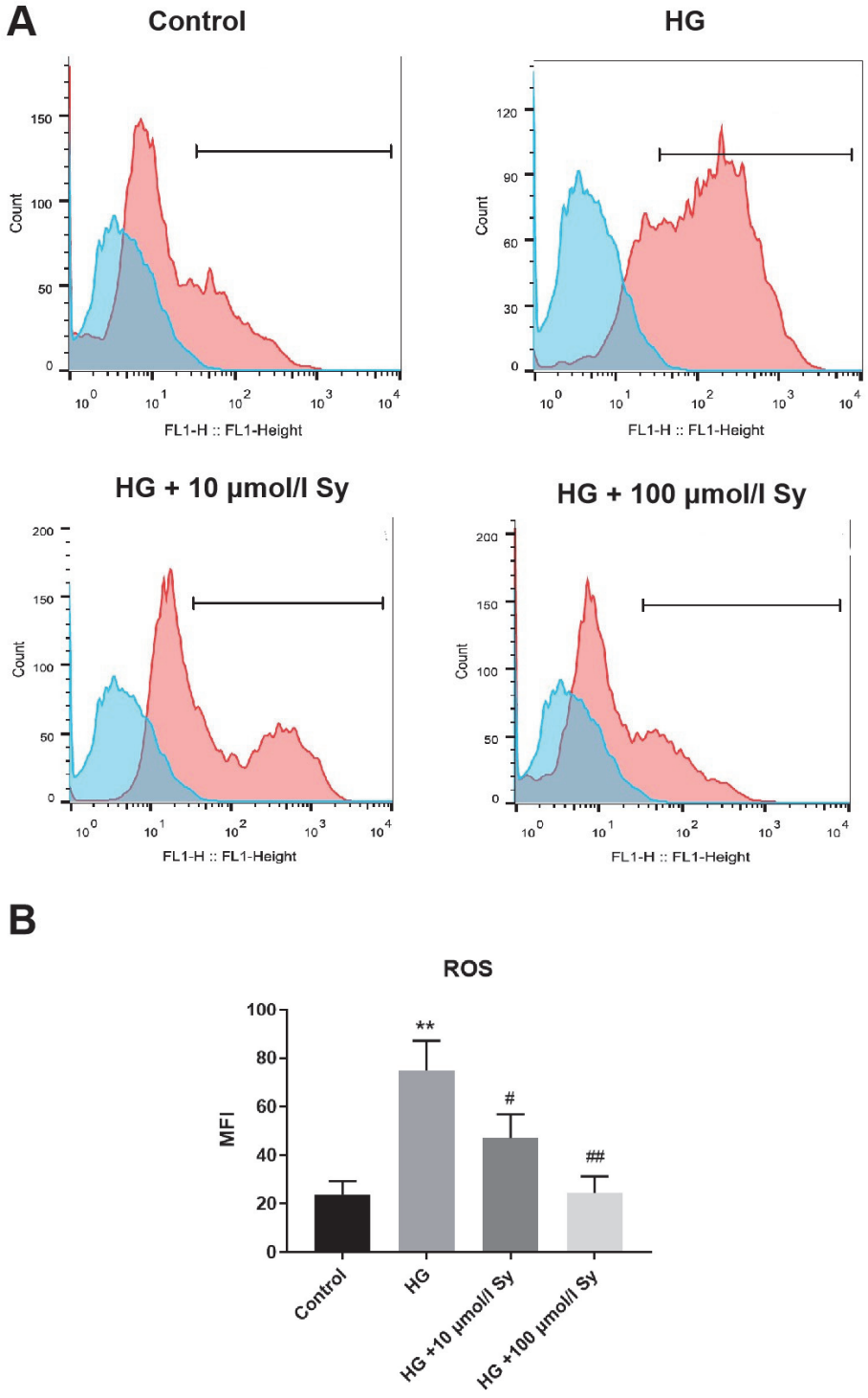

Fig. 6. The level of ROS in differentially treated BMSCs. (**A**) The ROS in the cells from the control, HG, HG + 10 μmol/l Sy, and HG + 100 μmol/l Sy groups were measured using Flow cytometry. (**B**) Summarized results showing the levels of ROS in cells. ** $P < 0.01$ compared to control cells; # and ## represent $P < 0.05$ and $P < 0.01$ compared to HG group of cells, respectively. MFI: Mean fluorescence intensity.

Syringin inactivates the p53/p21 and JAK2/STAT3 signaling pathways

As showed in Fig. 7, we observed significantly higher levels of p21 and p53 in the cells treated with HG, compared to the control cells (** $P < 0.01$). Moreover, the levels of p-JAK2, JAK2, p-STAT3, and STAT3 were also significantly higher in the HG group ($P < 0.05$) (Fig. 7A–G). Meanwhile, we found Syringin at both 10 μmol/l and 100 μmol/l significantly decreased p53 and p21 levels (# $P < 0.05$~ ## $P < 0.01$) (Fig. 7A–G). Finally, we detected whether JAK2/STAT3 signaling is involved. Accordingly, we observed that Syringin at 100 μmol/l significantly decreased p-JAK2, JAK2, p-STAT3, STAT3 levels (# $P < 0.05$~ ## $P < 0.01$) (Fig. 7A–G). Although the levels of p-JAK2 and p-STAT3 in the HG + 10 umol/l Sy group did not show significant differences when compared with the HG group, the total JAK2 and STAT3 levels were lower in the HG + 10 umol/l group compared with the HG group (Fig. 7A–G). These observations suggest that Syringin exerted its anti-senescence effect through regulating the p53/p21 and JAK2/STAT3 signaling.

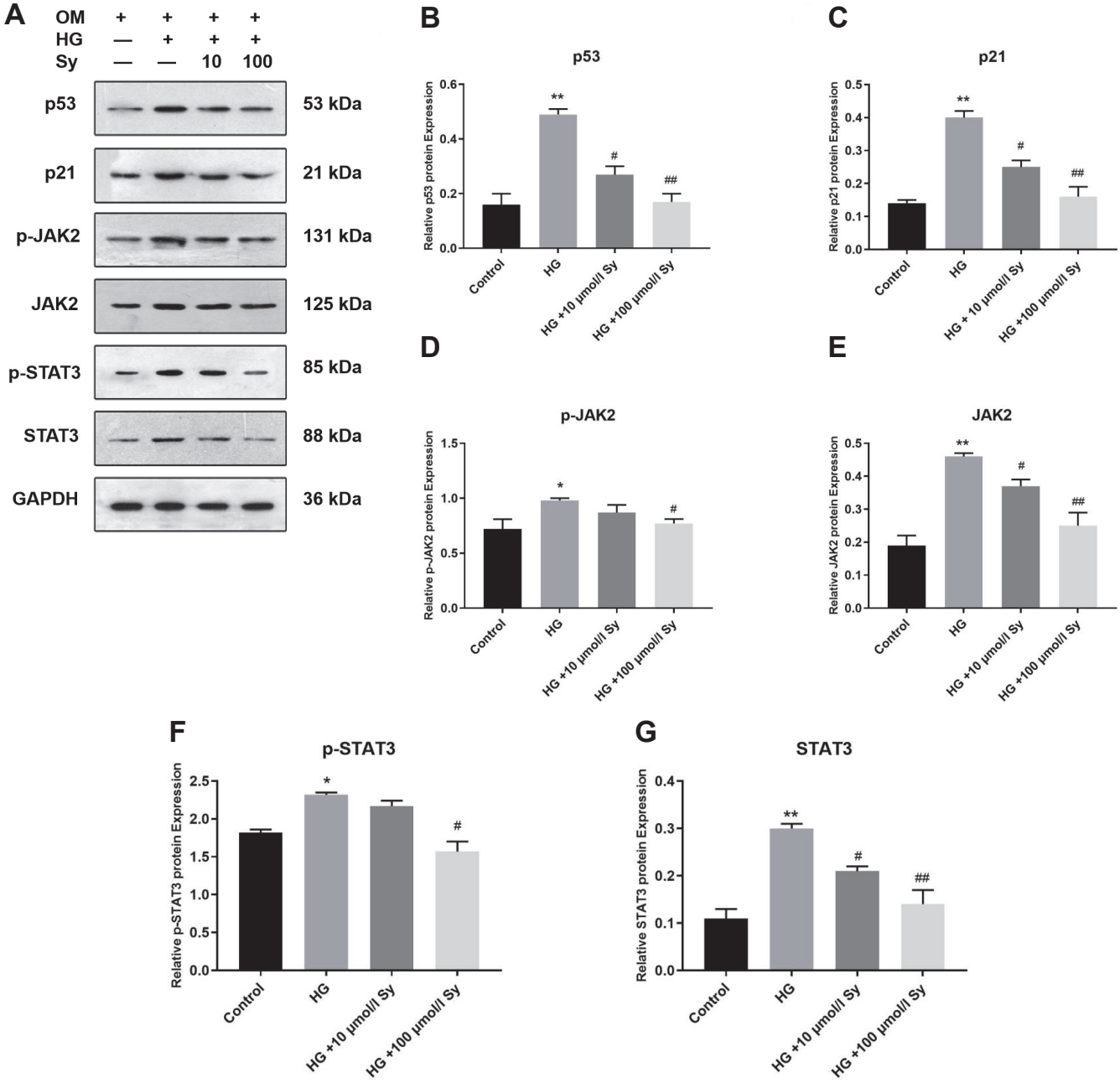

Fig. 7. (**A**) The protein levels of p21, p53, STAT3, p-STAT3, JAK2, and p-JAK2 in cells from the control, HG, HG + 10 μmol/l Sy, and HG + 100 μmol/l Sy groups. (**B–G**) Comparison of p53, p21, p-JAK2, JAK2, p-STAT3, and STAT3 protein levels among the four groups. * and ** represents $P < 0.05$ and $P < 0.01$ compared to control cells respectively; # and ## represents $P < 0.05$ and $P < 0.01$ compared to HG group of cells, respectively.

Discussion

Osteoporosis is a degenerative disease that has a high incidence and is almost impossible to completely cure. The disease burden is even greater in China because of the large population. In China and many other Asian countries, osteoporosis has been treated with TCM for millennia. As research continues, many of the components of TCM have been shown to have proven efficacy. For example, polysaccharides, which are widely found in many anti-osteoporosis TCM herbs, have been shown to achieve antioxidant, anti-fatigue, and balanced bone resorption functions in osteoporosis treatment (Lei et al., 2021). Zhang et al. (2016) collected the worldwide literature on the treatment of osteoporosis with TCM formulas from 1989 to 2015 and found that 23 TCM formulas, which were commonly used to treat osteoporosis, showed significant anti-osteoporotic effects in humans and animals. Although there is great potential for the development of antiosteoporotic drugs, many therapeutic mechanisms of TCM need to be elucidated. Findings from our current study indicated that BMSCs treated with high glucose (HG) together with Syringin (10 μmol/l or 100 μmol/l) had better proliferation and osteogenesis potential than the HG-induced senescence group. Further, mechanistic study suggested that Syringin suppresses the generation of ROS, inhibits the G1 cell cycle arrest, and reduces SA-β-Gal expression via regulating the activation of p53/p21 and JAK2/STAT3 signaling.

Both osteogenesis and the cellular senescence of BMSCs play great roles in bone formation. It has been reported that the senescence of BMSCs was significantly associated with declined cellular function and osteogenesis potential (Zhou et al., 2008). In line with this observation, our results showed that BMSCs also displayed senescent phenotype after HG treatment, along with declined cellular functions, such as impaired proliferation, G1 cell cycle arrest, decreased osteogenesis potential, high production of ROS, high percentage of SA-β-gal positive cells, and activation of p53/p21 and JAK2/STAT3 signaling. These phenomena indicated that most BMSCs become senescent after high glucose stimulation.

Previous studies have shown that Syringin could alleviate osteoporosis and diabetes as mentioned above. In our study, we demonstrated that Syringin decreased HG-induced senescence-associated phenotype by decreasing the percentage of SA-β-gal positive cells, increasing the cell proliferation rate and osteogenesis potential, and upregulated the percentage of G1 cell cycle. Additional studies have also shown that Syringin could selectively eliminate oxidative stress and thus act as an effective antioxidant (Shen et al., 2020).

ROS consists of a group of highly reactive molecules, such as non-radical oxygen derivatives, hydroxyl radical, and free oxygen radicals. It is significantly involved in the activation of signaling pathways, cell components, and molecular species, in a concentration-dependent manner (Davalli et al., 2016), and regulates many biological processes such as cell proliferation, cell cycle arrest, and cell death (Dimozi et al., 2015). Studies have shown that excessive ROS gathering is able to activate p53 and p21, leading to the phenotype of senescence (Adams, 2009). Increased production of ROS was observed in the senescent cells induced by oxidative stress (de Almeida et al., 2017). Meanwhile, the ROS/p53/p21 signaling pathway was identified as a key regulator during the cell senescence in BMSCs (Hu et al., 2017; Zhou et al., 2017). In our study, it was observed that the addition of Syringin dramatically decreased the production of ROS in cells and significantly decreased the p21 and p53 levels, which were increased by HG treatment in BMSCs. Our data suggest that Syringin treatment relieved BMSCs senescence caused by HG by regulation of ROS/p53/p21 signaling pathway.

The activation of JAK/STAT signaling pathway was closely associated with the inflammatory factors production. The signaling pathways mediated by the JAK/STAT axis was commonly expressed and involved in many biological processes, such as inflammatory response, immune response, cell growth, and cell apoptosis (Dodington et al., 2018). Through controlling the expression and secretion of inflammatory regulators, such as MCP1, PAI1, IL8, and IL6, STAT3 could directly affect the initiation and duration of the inflammatory microenvironment (Dodington et al., 2018). Another study also reported that JAK2/STAT3 axis was significantly involved in the mediation of senescence-associated secretory phenotype (SASP) factors, and subsequently participated in the processes of ovariectomized BMSC senescence. An enhanced osteogenic differentiation, reduced SASP factor expression, and alleviated senescence were observed after the inhibition of JAK (Wu et al., 2020). In addition, p53/p21 has been shown to be a direct target of STAT3 (Chen et al., 2016). However, whether the STAT3 and JAK2 mediated signaling pathway was associated with the senescence caused by HG in BMSCs is rarely reported. In our study, we found the total JAK2/STAT3 as well as p-JAK2 and p-STAT3 were all increased in the HG group in comparison with the control. Syringin with both 10 and 100 μmol/l inhibits JAK2/STAT3 signaling.

Conclusion

Syringin reversed high glucose-caused osteoporosis in BMSCs by altering the arrest of G0/G1 cell cycle, decreasing SA-β-gal activity and ROS production, increasing cell growth, and promoting osteogenesis potential. The mechanism within may be correlated with the involvement of p53 and p21, as well as JAK/STAT3 signaling. Further *in vivo* studies are needed to confirm the findings.

Ethical aspects and conflict of interest

The authors have no conflict of interest to declare.

References

Adams PD (2009). Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. Mol Cell 36(1): 2–14. DOI: 10.1016/j.molcel.2009.09.021.

An J, Yang H, Zhang Q, Liu C, Zhao J, Zhang L, Chen B (2016). Natural products for treatment of osteoporosis: The effects and mechanisms on promoting osteoblast-mediated bone formation. Life Sci 147: 46–58. DOI: 10.1016/j.lfs.2016.01.024.

Cai GP, Liu YL, Luo LP, Xiao Y, Jiang TJ, Yuan J, Wang M (2022). Alkbh1-mediated DNA N6-methyladenine modification regulates bone marrow mesenchymal stem cell fate during skeletal aging. Cell Prolif 55(2): e13178. DOI: 10.1111/cpr.13178.

Cao G, Hu S, Ning Y, Dou X, Ding C, Wang L, et al. (2024). Traditional Chinese medicine in osteoporosis: from pathogenesis to potential activity. Front Pharmacol 15: 1370900. DOI: 10.3389/fphar.2024.1370900.

Chang TC, Hsu MF, Wu KK (2015). High glucose induces bone marrow-derived mesenchymal stem cell senescence by upregulating autophagy. PloS One 10(5): e0126537. DOI: 10.1371/journal.pone.0126537.

Chen J, Xu T, Zhu D, Wang J, Huang C, Lyu L, et al. (2016). Egg antigen p40 of *Schistosoma japonicum* promotes senescence in activated hepatic stellate cells by activation of the STAT3/p53/p21 pathway. Cell Death Dis 7(7): e2315. DOI: 10.1038/cddis.2016.228.

Cheng T, Hou JL, Han ZY, Geng XL, Zhang YC, Fan KY, et al. (2024). Genetically determined type 1 diabetes mellitus and risk of osteoporosis. Exp Gerontol 191: 112434. DOI: 10.1016/j.exger.2024.112434.

Chu DT, Phuong TNT, Tien NLB, Tran DK, Thanh VV, Quang TL, et al. (2020). An Update on the Progress of Isolation, Culture, Storage, and Clinical Application of Human Bone Marrow Mesenchymal Stem/Stromal Cells. Int J Mol Sci 21(3): 708. DOI: 10.3390/ijms21030708.

Davalli P, Mitic T, Caporali A, Lauriola A, D'Arca D (2016). ROS, Cell Senescence, and Novel Molecular Mechanisms in Aging and Age-Related Diseases. Oxid Med Cell Longev 2016: 3565127. DOI: 10.1155/2016/3565127.

de Almeida AJPO, Ribeiro TP, de Medeiros IA (2017). Aging: Molecular Pathways and Implications on the Cardiovascular System. Oxid Med Cell Longev 2017: 7941563. DOI: 10.1155/2017/7941563.

Dimozi A, Mavrogonatou E, Sklirou A, Kletsa D (2015). Oxidative stress inhibits the proliferation, induces premature senescence and promotes a catabolic phenotype in human nucleus pulposus intervertebral disc cells. Eur Cell Mater 30: 89–103. DOI: 10.22203/ecm.v030a07.

Dodington DW, Desai HR, Woo M (2018). JAK/STAT – Emerging Players in Metabolism. Trends Endocrinol Metab 29(1): 55–65. DOI: 10.1016/j.tem.2017.11.001.

Eleftheriadis T, Pissas G, Filippidis G, Efthymiadi M, Liakopoulos V, Stefanidis I (2022). Dapagliflozin Prevents High-Glucose-Induced Cellular Senescence in Renal Tubular Epithelial Cells. Int J Mol Sci 23(24): 16107. DOI: 10.3390/ijms232416107.

Estrada JC, Torres Y, Benguría A, Dopazo A, Roche E, Carrera-Quintanar L, et al. (2013). Human mesenchymal stem cell-replicative senescence and oxidative stress are closely linked to aneuploidy. Cell Death Dis 4(6): e691. DOI: 10.1038/cddis.2013.211.

Ferraro F, Lymperi S, Méndez-Ferrer S, Saez B, Spencer JA, Yeap BY, et al. (2011). Diabetes impairs hematopoietic stem cell mobilization by altering niche function. Sci Transl Med 3(104): 104ra101. DOI: 10.1126/scitranslmed.3002191.

Hu D, Cao S, Zhang G, Xiao Y, Liu S, Shang Y (2017). Florfenicol-induced Mitochondrial Dysfunction Suppresses Cell Proliferation and Autophagy in Fibroblasts. Sci Rep 7(1): 13554. DOI: 10.1038/s41598-017-13860-9.

Huang C, Wang Y (2022). Downregulation of METTL14 improves postmenopausal osteoporosis via IGF2BP1 dependent posttranscriptional silencing of SMAD1. Cell Death Dis 13(11): 919. DOI: 10.1038/s41419-022-05362-y.

Itahana K, Campisi J, Dimri GP (2007). Methods to detect biomarkers of cellular senescence: the senescence-associated beta-galactosidase assay. Methods Mol Biol 371: 21–31. DOI: 10.1007/978-1-59745-361-5_3.

Khosla S, Samakkarnthai P, Monroe DG, Farr JN (2021). Update on the pathogenesis and treatment of skeletal fragility in type 2 diabetes mellitus. Nat Rev Endocrinol 17(11): 685–697. DOI: 10.1038/s41574-021-00555-5.

Kojima H, Kim J, Chan L (2014). Emerging roles of hematopoietic cells in the pathobiology of diabetic complications. Trends Endocrinol Metab 25(4): 178–187. DOI: 10.1016/j.tem.2014.01.002.

Kou Y, Rong X, Tang R, Zhang Y, Yang P, Liu H, et al. (2023). Eldecalcitol prevented OVX-induced osteoporosis through inhibiting BMSCs senescence by regulating the SIRT1-Nrf2 signal. Front Pharmacol 14: 1067085. DOI: 10.3389/fphar.2023.1067085.

Kumar P, Nagarajan A, Uchil PD (2018). Analysis of Cell Viability by the MTT Assay. Cold Spring Harb Protoc 2018(6). DOI: 10.1101/pdb.prot095505.

Lei SS, Su J, Zhang Y, Huang XW, Wang XP, Huang MC, et al. (2021). Benefits and mechanisms of polysaccharides from Chinese medicinal herbs for anti-osteoporosis therapy: A review. Int J Biol Macromol 193(Pt B): 1996–2005. DOI: 10.1016/j.ijbiomac.2021.11.030.

Liu J, Zhang Z, Guo Q, Dong Y, Zhao Q, Ma X (2018). Syringin prevents bone loss in ovariectomized mice via TRAF6 mediated inhibition of NF-κB and stimulation of PI3K/AKT. Phytomedicine 42: 43–50. DOI: 10.1016/j.phymed.2018.03.020.

Luo M, Zhao Z, Yi J (2023). Osteogenesis of bone marrow mesenchymal stem cell in hyperglycemia. Front Endocrinol 14: 1150068. DOI: 10.3389/fendo.2023.1150068.

Ma H, Bell KN, Loker RN (2020). qPCR and qRT-PCR analysis: Regulatory points to consider when conducting biodistribution and vector shedding studies. Mol Ther Methods Clin Dev 20: 152–168. DOI: 10.1016/j.omtm.2020.11.007.

Mao J, Tan L, Tian C, Wang W, Zhang H, Zhu Z, Li Y (2023). Hepatoprotective effect of syringin combined with costunolide against LPS-induced acute liver injury in L-02 cells via Rac1/AKT/NF-κB signaling pathway. Aging 15(21): 11994–12020. DOI: 10.18632/aging.205161.

Martiniakova M, Biro R, Penzes N, Sarocka A, Kovacova V, Mondockova V, Omelka R (2024). Links among Obesity, Type 2 Diabetes Mellitus, and Osteoporosis: Bone as a Target. Int J Mol Sci 25(9): 4827. DOI: 10.3390/ijms25094827.

Niu HS, Hsu FL, Liu IM, Cheng JT (2007). Increase of beta-endorphin secretion by syringin, an active principle of *Eleutherococcus senticosus*, to produce antihyperglycemic action in type 1-like diabetic rats. Horm Metab Res 39(12): 894–898. DOI: 10.1055/s-2007-993154.

Ogrodnik M (2021). Cellular aging beyond cellular senescence: Markers of senescence prior to cell cycle arrest *in vitro* and *in vivo*. Aging Cell 20(4): e13338. DOI: 10.1111/acel.13338.

Pozarowski P, Darzynkiewicz Z (2004). Analysis of cell cycle by flow cytometry. Methods Mol Biol 281: 301–311. DOI: 10.1385/1-59259-811-0:301.

Qian Q, Pan J, Yang J, Wang R, Luo K, Wu Z, et al. (2024). Syringin: a naturally occurring compound with medicinal properties. Front Pharmacol 15: 1435524. DOI: 10.3389/fphar.2024.1435524.

Reimann M, Lee S, Schmitt CA (2024). Cellular senescence: Neither irreversible nor reversible. J Exp Med 221(4): e20232136. DOI: 10.1084/jem.20232136.

Shah VN, Shah CS, Snell-Bergeon JK (2015). Type 1 diabetes and risk of fracture: meta-analysis and review of the literature. Diabet Med 32(9): 1134–1142. DOI: 10.1111/dme.12734.

Shen Z, Yang C, Zhu P, Tian C, Liang A (2020). Protective effects of syringin against oxidative stress and inflammation in diabetic pregnant rats via TLR4/MyD88/NF-κB signaling pathway. Biomed Pharmacother 131: 110681. DOI: 10.1016/j.biopha.2020.110681.

Singh VK, Thakur DC, Rajak N, Giri R, Garg N (2024). Immunomodulatory potential of bioactive glycoside syringin: a network pharmacology and molecular modeling approach. J Biomol Struct Dyn 42(8): 3906–3919. DOI: 10.1080/07391102.2023.2216299.

Singh VK, Thakur DC, Rajak N, Mishra A, Kumar A, Giri R, Garg N (2023). The multi-protein targeting potential of bioactive syringin in inflammatory diseases: using molecular modelling and in-silico analysis of regulatory elements. J Biomol Struct Dyn 26: 1–12. DOI: 10.1080/07391102.2023.2273440.

Wang L, Li Y, Gu J, Xiao L, Wang J (2024). Knowledge, Awareness and Perception towards Osteoporosis Risk in China: A Systematic Review. Iran J Public Health 53(5): 1009–1020. DOI: 10.18502/ijph.v53i5.15581.

Wu W, Fu J, Gu Y, Wei Y, Ma P, Wu J (2020). JAK2/STAT3 regulates estrogen-related senescence of bone marrow stem cells. J Endocrinol 245(1): 141–153. DOI: 10.1530/JOE-19-0518.

Xue WJ, He CF, Zhou RY, Xu XD, Xiang LX, Wang JT, et al. (2022). High glucose and palmitic acid induces neuronal senescence by NRSF/REST elevation and the subsequent mTOR-related autophagy suppression. Mol Brain 15(1): 61. DOI: 10.1186/s13041-022-00947-2.

Zhai Z, Chen W, Hu Q, Wang X, Zhao Q, Tuerxunyiming M (2020). High glucose inhibits osteogenic differentiation of bone marrow mesenchymal stem cells via regulating miR-493-5p/

ZEB2 signalling. J Biochem 167(6): 613–621. DOI: 10.1093/jb/mvaa011.

Zhang ND, Han T, Huang BK, Rahman K, Jiang YP, Xu HT, et al. (2016). Traditional Chinese medicine formulas for the treatment of osteoporosis: Implication for antiosteoporotic drug discovery. J Ethnopharmacol 189: 61–80. DOI: 10.1016/j.jep.2016.05.025.

Zheng L, Li M, Li H (2024). High Glucose Promotes and Aggravates the Senescence and Dysfunction of Vascular Endothelial Cells in Women with Hyperglycemia in Pregnancy. Biomolecules 14(3): 329. DOI: 10.3390/biom14030329.

Zhou S, Greenberger JS, Epperly MW, Goff JP, Adler C, Leboff MS, Glowacki J (2008). Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell 7(3): 335–343. DOI: 10.1111/j.1474-9726.2008.00377.x.

Zhou Z, Yin Y, Chang Q, Sun G, Lin J, Dai Y (2017). Downregulation of B-myb promotes senescence via the ROS-mediated p53/p21 pathway, in vascular endothelial cells. Cell Prolif 50(2): e12319. DOI: 10.1111/cpr.12319.