Glucocorticoid promotes osteoblast viability by activating autophagy via the SGK1/FOXO3a signal pathway

Xianghua Yu (✉ yuxianghua@126.com)

Shang Minhang District Dental Disease Prevention and Control Institute <https://orcid.org/0000-0001-6340-3235>

Xiaoming Xu

Minhang District Dental Disease Prevention and Control Institute

Shengxiang Zhang

Binzhou Medical College: Binzhou Medical University

Research Article

Keywords: Autophagy, Dexamethasone, Osteoblast, SGK1, FOXO3a

Posted Date: July 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-716576/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Autophagy is important for cellular survival under numerous stimuli. Previous studies have demonstrated that endogenous glucocorticoids (GCs) could induce autophagy, contribute to bone metabolism and development under physiological condition, but the precise mechanisms involved are unknown.

Methods: Mouse embryonic osteoblastic precursor cells, MC3T3-E1 cells, were treated with low dose of GCs (10^{-8} M dexamethasone, Dex) and/or autophagy inhibitor, 3-methyladenine (3-MA) for different time periods. Gene interference with serum- and glucocorticoid induced kinase-1 (SGK1) was performed before above stimulations. After that, CCK-8 assay was performed to determine cell viability and the flow cytometry was performed to detect the apoptosis rates of the osteoblast cells. The expressions of SGK1, phosphorylated-FOXO3a (p-FOXO3a)/FOXO3 and autophagy-related proteins were detected with the western blotting.

Results: A low dose (10^{-8} M) of Dex induced a significant time-dependent increase in the expression and activation of SGK1 in MC3T3-E1 cells, accompanied by an increase in cell viability and decrease in cell apoptosis. 3-MA significantly inhibited the viability-promoting effect of Dex. Moreover, we found that Dex increased LC3II and Beclin-1 levels and decreased SQSTM/p62 level in a time-dependent manner, which could be attenuated by pretreatment with 3-MA. Transfection of MC3T3-E1 cells exposed to Dex with shRNA-SGK1 resulted in a significant reduction in cell viability, and an increase in apoptosis. 3-MA further aggravated the effects of SGK1 inhibition on cell viability and apoptosis. Knocking down SGK1 before Dex exposure significantly downregulated p-FOXO3a/FOXO3, suppressed LC3II and Beclin-1 levels and increased SQSTM/p62 levels in MC3T3-E1 cells, which could be amplified by 3-MA.

Conclusions: A low dose of GCs increased osteoblast viability by activating autophagy via SGK1/FOXO3a pathway.

Background

With the increasing proportion of the elderly in the population, osteoporosis has become an important global health problem now. Osteoporosis is a progressive and systemic bone metabolic disease characterized by osteopenia, bone fragility and increased fracture tendency [1]. Osteoblasts play an important role in bone remodeling by producing extracellular matrix and promoting its mineralizations. Abnormal function of osteoblasts is an important cause of osteoporosis [2]. Improving osteoblasts viability and function have the potential applications in therapy of osteogenesis.

Autophagy is a highly conserved lysosome-dependent degradation pathway for recycling damaged cytosolic proteins and organelles to facilitate cellular homeostasis and promote cell survival under stress conditions, such as nutrient deprivation and hypoxia [3]. The induction of autophagy was recently shown to be useful for maintaining bone stability [4, 5]. Endogenous glucocorticoids (GCs) could induce autophagy, contribute to bone metabolism and development under physiological condition [6, 7].

Targeting autophagy and homeostasis will be a potential strategy to protect osteoblasts function and retard senescence. However, the role of autophagy during physiological dose of GCs-afforded osteoblasts protection and the precise mechanisms involved are poorly understood.

Serum- and glucocorticoid induced kinase-1 (SGK1) is a serine/threonine kinase initially identified as immediate early gene transcriptionally regulated by serum and glucocorticoids. SGK1 is remarkable in being activated at both the transcriptional and posttranslational levels by a huge number of extracellular signals, such as hyperosmotic stress, ultraviolet radiation, heat shock, oxidative stress and hypoxia [8]. SGK1 supports cell survival and cell migration, a prerequisite of tissue repair. Moreover, SGK1 negatively regulates the pro-apoptotic transcription factor forkhead box O3a (FOXO3a) via phosphorylation and exclusion from the nucleus [9]. Previous study demonstrated that the SGK1/FOXO3a pathway promoted cell survival by inhibiting cell cycle arrest and apoptosis [10]. Recently, SGK1 is known to act as a switch for autophagy homeostasis. Xie et al. showed that SGK1 overexpression promoted the autophagy flux and enhanced the protective effect mediated by hypoxic preconditioning [11].

Considering above evidences, we wondered whether the SGK1/FOXO3a signaling pathway is involved in the protection afforded by low dose of dexamethasone (Dex) via regulating autophagy. In this study, we intend to investigate whether SGK1 is implicated in the regulation of cell viability in osteoblastic precursor cells under low dose of Dex incubation and explore the potential mechanism.

Material And Methods

Cell culture and transfection

The murine embryonic osteoblastic precursor cell line MC3T3-E1, was purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; ThermoFisher Scientific), 2 mmol/L glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37°C in a humidified incubator containing 5% CO₂. Cells were exposed to 10⁻⁸ M Dex, an inducer which commonly used in osteoblasts differentiation for different amounts of time. MC3T3-E1 cells maintained in medium without Dex exposure were used as the normal control. To suppress autophagy, 5 mM 3-methyladenine (3-MA; Sigma-Aldrich, St. Louis, MO, USA) was added to the medium 1 h before each experiment.

The murine expression vectors PGpU6/GFP//Neo-Control, PGpU6/GFP//Neo-SGK1 (shRNA-SGK1) were purchased from or constructed by GenePharma (Shanghai, China). Briefly, the MC3T3-E1 cells were seeded in 6-well plates with serum-free DMEM and then subjected to a mixture of shRNA and Lipofectamine 3000 (ThermoFisher Scientific) reagent. After incubation for 48 h, the medium was changed, and the cells were incubated with fresh medium for further experiments.

Cell viability analysis

Cell viability was detected with a Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. The optical density (OD) was measured with a microplate reader (Thermo Fisher Scientific) at 450 nm. The percentage of living cells was calculated based on the ratio of absorbance of the experimental group to that of the control group.

Flow cytometry analysis of apoptosis

The cells were collected and stained with the Annexin-V/PI apoptosis assay kit (Thermo Fisher Scientific) according to the manufacturer's instructions and were analyzed using a FACS Calibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Ten thousand cells from the sample were scanned, and the data were analyzed using Cell Quest software (BD Biosciences). Annexin V+/PI- cells were considered early apoptotic cells.

Quantitative RT-PCR

Total RNA was extracted from cells (2×10^6) and rat renal tissues (0.1 mg) using TRIzol (ThermoFisher, Hudson, NH, USA) according to the manufacturer's protocol. Aliquots (5 mg) of RNA were reverse transcribed to cDNA using the Superscribe First-Strand Synthesis System (ThermoFisher, Hudson, NH, USA). qRT-PCR was conducted according to the manufacturer's instructions (ThermoFisher, Hudson, NH, USA). Reactions were run on a real-time PCR system (ABI PRISM 7700; Applied Biosystems, Foster, CA). Gene expression was detected with the SYBR Green RT-PCR Kit (ThermoFisher, Hudson, NH, USA), and relative gene expression was determined by normalization to GAPDH and the $2^{-\Delta\Delta CT}$ method.

Western blot analysis

The treated MC3T3-E1 cells were collected and lysed with RIPA buffer supplemented with complete protease inhibitor cocktail tablets. The protein concentration was measured using the DC Protein Assay Kit (Bio-Rad Laboratories, Shanghai, China). Equal amounts of protein were separated on SDS-polyacrylamide gels and transferred onto PVDF membranes (Millipore, Billerica, MA, USA). The membranes were then incubated overnight at 4°C with antibodies against SGK1, phospho-SGK1, FOXO3a, phospho-FOXO3a, Beclin-1 and cleaved caspase-3 (1:1000; Cell Signaling, Danvers, MA, USA); LC3B (1:500; Novus, Littleton, CO, USA); SQSTM1/p62 (1:1000, Abcam, Shanghai, China); and β -actin (1:5000; Sigma-Aldrich, St. Louis, MO, USA) respectively. Next, the membranes were blotted with HRP-conjugated anti-rabbit or anti-mouse IgG antibody (1:5000, Jackson, West Grove, PA, USA). The protein bands were visualized using ECL Plus (Amersham) according to the manufacturer's instructions and developed on film. The relative density was quantified using ImageJ software.

Statistical analysis

All the experiments were conducted in triple and all data are presented as the mean $\pm$ standard deviation (SD). Statistical analysis was performed using Student's t test or one-way ANOVA with GraphPad Prism 9.0. $P < 0.05$ was considered as statistically significant.

Results

Dexamethasone dynamically induces SGK1 expression and activation in MC3T3-E1 cells

We investigated the effect of different durations of low-dose Dex exposure on SGK1 expression and activation using quantitative RT-PCR (Fig. 1A) or western blot analysis (Fig. 1B). Treating MC3T3-E1 cells with 10^{-8} M Dex induced a significant time-dependent increase in the expression of SGK1 both at mRNA levels (Fig. 1A) and protein levels (Fig. 1B). Interestingly, this low-dose Dex induced SGK1 expression and phosphorylation in the MC3T3-E1 cells were very fast, which began to increase 1h after Dex stimulation, peaked at 3h, and then decreased gradually until 24h after Dex exposure (Fig. 1). These results demonstrate that low-dose Dex exposure promotes both the expression and activation of the SGK1 in MC3T3-E1 cells.

Autophagy is involved in the effect of dexamethasone on MC3T3-E1 cells viability

To clarify the effect of different duration of low-dose Dex exposure on MC3T3-E1 cells viability and if the effect is associated with autophagy, MC3T3-E1 cells were pretreated with 3-MA, an autophagy inhibitor, before Dex incubation. The results showed that the viability of MC3T3-E1 cells treated with a low-dose of Dex (10^{-8} M) was increased at 24h (Fig. 2A). 3-MA significantly inhibited the viability-promoting effect of Dex for MC3T3-E1 cells (Fig. 2A). Meanwhile, the results of flow cytometry analysis showed that the decrease in apoptotic cells induced by low-dose Dex (10^{-8} M) was inhibited by 3-MA (Fig. 2B). Therefore, we speculate that the effect of low-dose Dex on MC3T3-E1 cells viability maybe closely related to autophagy.

Dexamethasone induce time-dependent autophagy in MC3T3-E1 cells

The primary method for measuring autophagic activity involves the detection of Beclin-1, SQSTM1/p62, and LC3I to LC3II processing. Different durations of low-dose Dex exposure led to time-dependent changes in autophagy in MC3T3-E1 cells, which peaked at 6h after Dex (10^{-8} M) incubation, as indicated by the expression of LC3II and Beclin-1. However, the protein levels of p62, a selective substrate of autophagy, were depleted since 6h after Dex stimulation (Fig. 3). 3MA pretreated cells showed a significantly time-dependent decrease in the expression of LC3II and Beclin-1 and an increase in the level of p62 compared to the Dex treated group (Fig. 3)

SGK1 promotes cell viability and inhibits the apoptosis of MC3T3-E1 cells exposed to dexamethasone

To further study the function of low-dose Dex (10^{-8} M) induced expression and phosphorylation of the SGK1 protein, we mediated the knockdown of SGK1 with shRNA and pharmacologically inhibited autophagy with 3-MA. Specifically, MC3T3-E1 cells transfected with the vector or SGK1 shRNA (shRNA-SGK1) for 48h were incubated with 3-MA for 1h and then treated with Dex for 24h. After these treatments, cell viability and apoptosis were determined. Transfection of MC3T3-E1 cells with shRNA-SGK1 resulted in a significant reduction in cell viability (Fig. 4A), and an increase in apoptosis compared to the Vector transfected cells under Dex culture (Fig. 4B). Moreover, Dex-induced MC3T3-E1 cell survival was attenuated by autophagy inhibitor 3-MA effectively (Fig. 4A) and 3-MA further aggravated the effects of SGK1 inhibition on cell viability and apoptosis (Fig. 4A, 4B).

SGK1 promotes autophagy in MC3T3-E1 cells exposed to dexamethasone via FOXO3a

Studies have shown that SGK1 is up-regulated by several types of cell stress and it supports cellular survival, cell migration, and wound healing through multiple signaling pathways. FOXO3a is an important downstream target of SGK1 [22]. To further explore the mechanism of Dex induced autophagy, we examined the SGK1/FOXO3a pathway upon Dex exposure by western blot. We measured the phosphorylated levels of FOXO3a and autophagy associated proteins levels after shRNA-SGK1 transfection with or without 3-MA treatment. We found that pretreatment with 3-MA alone before Dex incubation obviously decreased the expressions of phosphorylated-FOXO3a, autophagic associated protein LC3II and Beclin-1, and distinctly increased the expression of p62 (Fig. 5).

Furthermore, inhibition of SGK1 significantly decreased FOXO3a phosphorylation, LC3II and Beclin-1 levels, and promoted the expression of p62 in response to Dex. 3-MA further reduced the levels of phosphorylated-FOXO3a, LC3II and Beclin-1, improved the level of p62 under Dex incubation with SGK1 inhibition (Fig. 5). These findings indicate that upon low-dose Dex exposure, SGK1 mediated autophagy may contribute to cell survival by phosphorylating and inactivating FOXO3a.

Discussion

In this study, we demonstrated that SGK1 was upregulated and activated in MC3T3-E1 cells incubated with low dose of Dex (10^{-8} M), an inducer which commonly used in osteoblasts differentiation. SGK1 knockdown decreased the cell viability, autophagic level and p-FOXO3/FOXO3a level induced by Dex exposure, confirming the protective role of SGK1 on osteoblasts differentiation. In addition, we showed that inhibition of autophagy aggravated the damaging effects of SGK1 knockdown as mentioned upon low dose of Dex exposure, suggesting that SGK1 might protect osteoblasts survival during the differentiation process by promoting autophagy and the FOXO3a signaling pathway.

Dexamethasone is a commonly used anti-inflammatory and an immunosuppressive drug used in clinical practice to treat a variety of diseases. Glucocorticoid-induced osteoporosis (GIO) has considered to be the most common form of secondary osteoporosis for the widely application of GCs. Interestingly, endogenous GCs are essential for the maintenance of bone homeostasis, whereas excess GCs may impede bone formation or facilitate bone resorption [12]. So, it is necessary to uncover the mechanism by which GCs maintains the balance of bone homeostasis under physiological condition and further develop effective strategies for treating or preventing GIO.

Bone homeostasis is a dynamic balance which mediated by osteoblasts, osteoclasts, bone marrow mesenchymal stem cells, and osteocytes. Recent studies demonstrated that different dose of GCs led to different bone cells fate [7, 13]. Osteoblasts, which are derived from mesenchymal stem cells, are mainly responsible for bone formation and development. Zhang S et al found a high dose of Dex ($\geq 10^{-6}$ M) accelerated osteoblasts apoptosis, while a low dose of Dex (10^{-8} M) increased osteoblasts viability in the

early stage [14]. This is consistent with our experimental results. Unfortunately the underlying mechanism is still complex and require further more investigation.

Autophagy is a highly conserved catabolic process that is essential for cell growth, survival, differentiation, development and homeostasis [15]. It has been identified that autophagy play a critical role in both physiological processes and numerous pathological conditions including inflammation, neurodegeneration, cancer and bone metabolic diseases [16]. Under physiological conditions, autophagy is responsible for the removal of damaged or excessive organelles, whereas under pathological conditions, autophagy helps in the redistribution of intracellular nutrients to meet the substance and energy requirement for survival. Despite an appropriate autophagy level is prerequisite for the maintenance of homeostasis and survival of cell, but the excessive activation of autophagy generally leads to a programmed cell death. Therefore, autophagy has been reported as a 'double-edged sword' [17]. Over the past ten years, many studies have suggested that autophagy in osteoblasts, plays a critical role in the bone homeostasis and GIO [18]. In the present study, we found that 10^{-8} M Dex induced osteoblast autophagy and maintained osteoblast viability. The positive effect of 10^{-8} M Dex on the occurrence of autophagy was confirmed, as reflected by the increased expression of beclin-1 and LC3II and the decreased expression of P62. Furthermore, our studies found that 10^{-8} M Dex increased LC3II and Beclin-1 levels and decreased SQSTM/p62 level in a time-dependent manner, which could be attenuated by pretreatment with autophagy inhibitor 3-MA, and 3-MA significantly inhibited the cell viability-promoting effect of Dex. Using knockdown of autophagy-

essential genes and osteoblasts-specific autophagy- deficient mice, researchers demonstrated that autophagy deficiency increased oxidative stress and reduced mineralization capacity, which showed that autophagy in osteoblasts is involved in bone homeostasis [19]. However the precise signal pathway of autophagy affecting the viability of osteoblasts exposed to low dose of GCs has rarely been reported.

SGK1 related to Akt (also called PKB), is a serine/threonine kinase that can be activated by PI3k, which is known for decades for its role in ion channel modulation and cell survival in response to stress stimuli. SGK1 participates in the regulation of transport, hormone release, tumor growth, neurodegeneration, cell proliferation, and apoptosis in multiple cell lines [20, 21]. In vitro studies indicated that protein levels and SGK1 activation were significantly stimulated by H_2O_2 exposure. Human proximal tubular cells with SGK1 inhibition were much more sensitive to H_2O_2 -induced oxidative stress injury than control group cells, as they exhibited increased apoptotic cell death and mitochondrial dysfunction [22]. Li J et al reported overexpression of SGK1 significantly attenuated A549 cell apoptosis and reduced the reactive oxygen species (ROS) generation induced by PM2.5 [23]. Our studies showed that treating MC3T3-E1 cells with 10^{-8} M Dex induced a significant time-dependent increase in the expression of SGK1 both at mRNA levels and protein levels, which began to increase 1h after Dex stimulation and peaked at 3 h. We supposed that SGK1 participates in the cellular survival of low dose Dex. So, we inhibited SGK1 expression using shRNA. The results showed that SGK1 knockdown weakened the promotional role of Dex on cell viability.

In addition to the above functions, SGK1 is now known to act as a switch for autophagy homeostasis. However, there is controversy regarding the effect of SGK1 on autophagy in different cell lines [24, 25]. Notably, we found that the effect of SGK1 on autophagy paralleled its significant increase in cell viability of MC3T3-E1 cells exposed to low dose of Dex. Moreover, inhibiting SGK1 significantly decreased the expression of autophagy markers LC3II and Beclin-1, promoted the expression of p62 and increase in apoptosis in MC3T3-E1 cells under Dex exposure compared to the control. 3-MA further aggravated the effects of SGK1 inhibition on cell viability and apoptosis.

FOXO3a, as a member of the forkhead transcription factor family, is a downstream target of SGK1. SGK1 phosphorylates FOXO3a leading to FOXO3a translocation from the nucleus to the cytoplasm and to the inhibition of FOXO3a-dependent transcription, thereby preventing FOXO3a from inducing apoptosis and/or cell cycle arrest [10]. FOXO3a has been reported to actively promote apoptosis by inducing Bcl-2 family members [26]. Similarly, Chen et al revealed that SGK1 activation significantly increased FOXO3a phosphorylation, and then inhibited the subsequent expression of Bcl-2 interacting mediator of cell death (Bim). SGK1 mediates the hypotonic protective effect against H₂O₂-induced apoptosis of rat basilar artery smooth muscle cells by inhibiting the FOXO3a/Bim signaling pathway [27]. Recent studies indicated that FOXO3a overexpression not only decreased HIF-1 α protein level but also inhibited HIF-1 α transcriptional activity, as evidenced by decreased expressions of HIF-1 target genes EPO, HO-1, and Bnip3 known to regulate autophagy [11, 28]. We show here that knocking down SGK1 before Dex exposure significantly downregulated phosphorylated-FOXO3a (p-FOXO3a)/FOXO3, suppressed LC3II and Beclin-1 levels and increased SQSTM/p62 levels in MC3T3-E1 cells, which could be amplified by 3-MA. The results revealed that a low dose of GCs increased osteoblasts viability by activating autophagy via SGK1/FOXO3a pathway.

Conclusions

Dexamethasone exerted a biphasic effect on the viability of osteoblast cells because of the different doses and treatment time. Our study indicated that low dose of dexamethasone could up-regulate SGK1, appropriately activate autophagy and promote cell survival by dephosphorylated inactivation of FOXO3a in MC3T3-E1 cells. We provide a new insight into autophagic regulatory mechanisms in early stage of low dose of Dex-mediated osteoblast differentiation and may identify novel therapeutic strategies to improve the osteoblasts function. Future studies in vivo genetic models will be required for clarifying the role of SGK1 in osteoblasts differentiation and its regulation of autophagy, which has emerged as a novel therapeutic target likely to attract considerable research attention in the coming years.

Abbreviations

GCs: glucocorticoids; Dex: dexamethasone; 3-MA: 3-methyladenine; SGK1: glucocorticoid induced kinase-1; FOXO3a: transcription factor forkhead box O3a; LC3: Little computer 3; DMEM: Dulbecco's Modified Eagle Medium; FBS: fetal bovine serum; CCK-8: Cell Counting Kit-8; OD: optical density; GIO: osteoporosis; ROS: reactive oxygen species.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

X.Y. designed the experiments and performed the experiments. X.Y. contributed to the cell culture and western blot analysis. S.Z. performed RT-PCR and cell viability analysis. X.X. was responsible for the statistical analysis. X.Y. wrote the paper. All authors have read and approved the final version of the manuscript submitted for publication.

Funding

This work was supported by the grants from the Minhang District Science and Technology Committee, Shanghai (No. 2021MHZ045, Xianghua Yu).

Availability of data and materials

The datasets generated/analyzed during the current study are available.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1 Minhang District Dental Disease Prevention and Control Institute, Shanghai, China. 2 Class 1, Grade 2017, School of Stomatology, Binzhou Medical University, Shandong, China.

References

1. Liu J, Curtis EM, Cooper C, Harvey NC. State of the art in osteoporosis risk assessment and treatment. J Endocrinol Invest. 2019;42:1149-64.
2. Lee WC, Guntur AR, Long F, Rosen CJ. [Energy Metabolism of the Osteoblast: Implications for Osteoporosis](#). Endocr Rev. 2017;38:255-66.

3. Kroemer G, Mariño G, Levine B. [Autophagy and the integrated stress response](#). Mol Cell. 2010;40:280-93.
4. Yin X, Zhou C, Li J, Liu R, Shi B, Yuan Q, Zou S. Autophagy in bone homeostasis and the onset of osteoporosis. Bone Res. 2019;7:28.
5. Pierrefite-Carle V, Santucci-Darmanin S, Breuil V, Camuzard O, Carle GF. Autophagy in bone: Self-eating to stay in balance. Ageing Res Rev. 2015;24:206-17.
6. Wang T, Liu X, He C. [Glucocorticoid-induced autophagy and apoptosis in bone](#). Apoptosis. 2020;25:157-68.
7. Jia J, Yao W, Guan M, Dai W, Shahnazari M, Kar R, Bonewald L, Jiang JX, Lane NE. Glucocorticoid dose determines osteocyte cell fate. FASEB J. 2011;25:3366-76.
8. Lang F, Cohen P. Regulation and physiological roles of serum- and glucocorticoid-induced protein kinase isoforms. Sci STKE. 2001;2001:re17.
9. Dehner M, Hadjihannas M, Weiske J, Huber O, Behrens J. Wnt signaling inhibits Forkhead box O3a-induced transcription and apoptosis through up-regulation of serum- and glucocorticoid-inducible kinase 1. J Biol Chem. 2008;283: 19201-10.
10. Brunet A, Park J, Tran H, Hu LS, Hemmings BA, Greenberg ME. [Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 \(FOXO3a\)](#). Mol Cell Biol. 2001;21:952-65.
11. Xie Y, Jiang D, Xiao J, Fu C, Zhang Z, Ye Z, Zhang X. Ischemic preconditioning attenuates ischemia/reperfusion-induced kidney injury by activating autophagy via the SGK1 signaling pathway. Cell Death Dis . 2018;9:338.
12. Hartmann K, Koenen M, Schauer S, Wittig-Blaich S, Ahmad M, Baschant U, Tuckermann JP. Molecular actions of glucocorticoids in cartilage and bone during health, disease, and steroid therapy. Physiol Rev. 2016;96:409-47.
13. Shi J, Wang L, Zhang H, Jie Q, Li X, Shi Q, Huang Q, Gao B, Han Y, Guo K, Liu J, Yang L, Luo Z. Glucocorticoids: Dose-related effects on osteoclast formation and function via reactive oxygen species and autophagy. Bone. 2015;79:222-32.
14. Zhang S, Liu Y, Liang Q. Low-dose dexamethasone affects osteoblast viability by inducing autophagy via intracellular ROS. Mol Med Rep. 2018 Mar;17(3):4307-4316.
15. Dikic I, Elazar Z. Mechanism and medical implications of mammalian autophagy. Nat Rev Mol Cell Biol. 2018;19:349-64.
16. Shapiro IM, Layfield R, Lotz M, Settembre C, Whitehouse C. Boning up on autophagy: the role of autophagy in skeletal biology. Autophagy. 2014;10:7-19.
17. Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. Science. 2004;306:990-5.
18. Nollet M, Santucci-Darmanin S, Breuil V, Al-Sahlanee R, Cros C, Topi M, Momier D, Samson M, Pagnotta S, Cailleteau L, Battaglia S, Farlay D, Dacquin R, Barois N, Jurdic P, Boivin G, Heymann D,

- Lafont F, Lu SS, Dempster DW, Carle GF, Pierrefite-Carle V. Autophagy in osteoblasts is involved in mineralization and bone homeostasis. *Autophagy*. 2014;10:1965-77.
19. Wang L, Heckmann BL, Yang X, Long H. Osteoblast autophagy in glucocorticoid-induced osteoporosis. *J Cell Physiol*. 2019;234:3207-15.
20. Lang F, Böhmer C, Palmada M, Seebohm G, Strutz-Seebohm N, Vallon V. (Patho) physiological significance of the serum- and glucocorticoid-inducible kinase isoforms. *Physiol Rev*. 2006;86:1151-78.
21. Leong ML, Maiyar AC, Kim B, O'Keeffe BA, Firestone GL. Expression of the serum- and glucocorticoid-inducible protein kinase, Sgk, is a cell survival response to multiple types of environmental stress stimuli in mammary epithelial cells. *J. Biol. Chem*. 2003;278:5871–82.
22. Jiang D, Fu C, Xiao J, Zhang Z, Zou J, Ye Z, Zhang X. [SGK1 Attenuates Oxidative Stress-Induced Renal Tubular Epithelial Cell Injury by Regulating Mitochondrial Function](#). *Oxid Med Cell Longev*. 2019;2019:2013594.
23. Li J, Zhou Q, Yang T, Li Y, Zhang Y, Wang J, Jiao Z. [SGK1 inhibits PM2.5-induced apoptosis and oxidative stress in human lung alveolar epithelial A549 cells](#). *Biochem Biophys Res Commun*. 2018;496:1291-5.
24. Maestro I, Boya P, Martinez A. [Serum- and glucocorticoid-induced kinase 1, a new therapeutic target for autophagy modulation in chronic diseases](#). *Expert Opin Ther Targets*. 2020;24:231-43.
25. Liu W, Wang X, Liu Z, Wang Y, Yin B, Yu P, Duan X, Liao Z, Chen Y, Liu C, Li X, Dai Y, Tao Z. [SGK1 inhibition induces autophagy-dependent apoptosis via the mTOR-Foxo3a pathway](#). *Br J Cancer*. 2017;117:1139-53.
26. Fu Z, Tindall DJ. FOXOs, cancer and regulation of apoptosis. *Oncogene*. 2008;27:2312-9.
27. Chen BY, Huang CC, Lv XF, Zheng HQ, Zhang YJ, Sun L, Wang GL, Ma MM, Guan YY. [SGK1 mediates the hypotonic protective effect against H2O2-induced apoptosis of rat basilar artery smooth muscle cells by inhibiting the FOXO3a/Bim signaling pathway](#). *Acta Pharmacol Sin*. 2020;41:1073-84.
28. Regmi S, Raut PK, Pathak S, Shrestha P, Park PH, Jeong JH. [Enhanced viability and function of mesenchymal stromal cell spheroids is mediated via autophagy induction](#). *Autophagy*. 2020;16:1-20.

Figures

Figure 1

Effect of dexamethasone (Dex) on the expression of SGK1. MC3T3-E1 cells were treated with Dex (10^{-8} M) for different time periods. (A) SGK1 mRNA levels were examined by Quantitative RT-PCR analysis. (B) Phosphorylated SGK1, total SGK1 protein levels were examined by western blot analysis. Relative mRNA and protein levels were normalized to β -actin. Data are presented as the mean $\pm$ SD (n=3). *P<0.05 vs. control, **P<0.01 vs. control.

Figure 2

Effect of the autophagy inhibitor 3-methyladenine (3-MA) on the viability of dexamethasone (Dex) treated cells. (A) MC3T3-E1 cells were treated with Dex (10-8M) or Dex (10-8M) +3-MA (5mM) for different time periods and cell viability was evaluated by CCK-8 assay. (B) Determination and quantitative analysis of apoptotic cells by Annexin V-propidium iodide FACS analysis. Data are presented as the mean $\pm$ SD (n=3). *P<0.05 vs. control, #P<0.05 vs. Dex group.

Figure 3

Effect of the autophagy inhibitor 3-methyladenine (3-MA) on the expression of autophagy associated proteins in dexamethasone (Dex) treated cells. MC3T3-E1 cells were treated with Dex (10⁻⁸M) or Dex (10⁻⁸M) +3-MA (5mM) for different time periods and autophagy associated protein levels were examined by western blot analysis. Relative protein levels were normalized to β-actin. Data are presented as the mean±SD (n=3). *P<0.05 vs. control, #P<0.05vs. Dex group.

Figure 4

Effect of SGK1 knockdown on the viability and apoptosis of dexamethasone (Dex) treated cells. MC3T3-E1 cells were transfected with vector control or SGK1 shRNA (shRNA-SGK1) were incubated with treated with 3-methyladenine (3-MA, 5mM) for 1h and then treated with DEX (10-8 M) for 24h. (A) Cell viability was assessed via the CCK-8 assay. (B) Cell apoptosis was determined by Annexin V-propidium iodide FACS analysis. Data are presented as the mean±SD (n=3). *P<0.05.

Figure 5

Effect of SGK1 knockdown on FOXO3a and autophagy associated proteins expression in dexamethasone (Dex) treated cells. MC3T3-E1 cells were transfected with vector control or SGK1 shRNA (shRNA-SGK1) were incubated with treated with 3-methyladenine (3-MA, 5mM) for 1h and then treated with Dex (10⁻⁸ M) for 6h. The expression levels of autophagy associated proteins were detected by western blot analysis. Data are presented as the mean $\pm$ SD (n=3). *P<0.05.