Lactoferrin promotes the autophagy activity during osteoblast formation via BCL2-Beclin1 signaling

Dianshan Ke1,3 · Xinwen Wang1 · Yinquan Lin1 · Shengwang Wei2

Received: 15 September 2021 / Accepted: 20 October 2021 / Published online: 29 October 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract
Background Lactoferrin, as the main component of milk, can maintain osteoblast formation, which is conducive to the prevention and treatment of osteoporosis. Lactoferrin also serves as an autophagy regulator, especially in osteoblasts. This study aimed to explore the significance of autophagy in osteoblast formation regulated by lactoferrin and the internal mechanism.

Methods and results In this study, we firstly explored the roles of lactoferrin in the autophagy activity of primary osteoblasts (LC3 transformation rate, autophagosome formation). Subsequently, we further investigated the effects of lactoferrin on the BCL2 expression and BCL2-Beclin1 complex. Ultimately, the significance of BCL2 overexpression and Beclin1 silencing on lactoferrin-regulated osteoblast autophagy and osteogenic parameters (ALP activity and mRNA expression of PCNA, Coll, BGLAP and OPN) was observed by gene processing, respectively. Our results showed that lactoferrin enhanced the autophagy activity of osteoblasts. Importantly, lactoferrin inhibited BCL2 expression and the co-immunoprecipitation of BCL2 and Beclin1 in osteoblasts. Moreover, lactoferrin-promoted autophagy and osteogenic parameters was reversed by BCL2 overexpression or Beclin1 silencing in osteoblasts.

Conclusions In conclusion, lactoferrin can inhibit BCL2 expression in osteoblasts, further enhancing Beclin1-dependent autophagy activation.

Keywords Lactoferrin · Osteoblasts · Autophagy · BCL2 · Beclin1

Introduction
Lactoferrin is a kind of iron-binding glycoprotein, which exists in milk, breast milk secretion and so on [1]. Lactoferrin can promote the osteoblast formation and subsequent osteogenesis, whereby improving pathological bone loss [2–4]. Chen et al. and Li et al. proved that bovine lactoferrin significantly improved bone mass and bone microstructure in ovariectomized rats [5, 6]. Furthermore, both bovine lactoferrin and human lactoferrin can efficiently promote the proliferation and osteogenic activity of osteoblast cell line, MC3T3-E1 [4, 7–10]. Lactoferrin can also improve the inhibition of osteogenesis induced by aging through IGF1 signaling [5]. Lactoferrin promotes the osteogenic activity of primary osteoblasts, MC3T3-E1 cells in vitro and C57BL/6J mice in vivo [6, 11]. Similar results were reported in other studies [12, 13].

Autophagy, as a highly conserved intracellular mechanism, maintains cell homeostasis by degrading damaged or aged organelles, breaking down dispensable macromolecules or pathogens, and releasing nutrients and energy. Autophagy also exerts a significant effect on the formation of osteoblasts. The knockdown of autophagic molecule, ATG5, can suppress the proliferation and differentiation of osteoblasts [14]. Moreover, autophagy activation also contributes to the osteogenic differentiation of human gingival mesenchymal stem cells [15]. Accordingly, the positive effect of autophagy has an important responsibility for osteoblast formation.
Other investigations have also clarified the similar theory [16–21]. Lactoferrin is an important regulator of autophagy. Most studies have elucidated the role of lactoferrin in promoting autophagy. Lactoferrin can improve the pathological cardiac hypertrophy of aging mouse heart by enhancing lysosome-dependent autophagy [22]. The protective effect of lactoferrin on hepatocytes is also related to the promotion of autophagy activity of damaged hepatocytes [23]. In addition, lactoferrin can induce the autophagy in human kidney proximal tubular cells and suppress oxidative stress-induced cell death by upregulating the autophagy activity [24]. Furthermore, lactoferrin suppresses renal fibrosis through the induction of autophagy [24]. At present, only one study has described the role of lactoferrin in osteoblast autophagy. However, the study only focused on the effect of lactoferrin on the autophagic activity of osteoblast precursors [12]. Therefore, there is still a lack of evidence for the direct effect of lactoferrin on osteoblast autophagy.

BCL2-Beclin1 signaling is a classic pathway of autophagy activation. BCL2 prevents Beclin1 from dissociating from BCL2-Beclin1 complex by conjugation, which leads to less Beclin1 entering autophagy flux and autophagy inactivation [25]. Under various stresses, BCL2-Beclin1 complex dissociates, leading to Beclin1-dependent autophagy activation [26–30]. Previous studies also lack the relevant reports regarding the regulatory effect of lactoferrin on BCL2 protein expression in osteoblasts. Therefore, the significance of BCL2-Beclin1 signaling in the autophagy and formation of osteoblasts regulated by lactoferrin is worthy of further study.

In this study, we explored the direct effect of lactoferrin on the autophagy of primary osteoblasts from rat calvaria. The experimental data showed that lactoferrin can enhance the autophagic activity of primary osteoblasts. In addition, a potential mechanism related to osteoblast formation dominated by lactoferrin, BCL2-Beclin1-autophagy signaling pathway, has also been revealed for the first time.

**Materials and methods**

**Cell isolation and culture**

Primary osteoblasts were prepared from the calvaria of Sprague Dawley (SD) rats (Animal center of Gem Pharmatech Co., Ltd; Nanjing, China). In short, the calvarias of rats (2 weeks after birth) were dissected, washed with PBS and digested in fresh 0.1% collagenase type II in alfa-minimal essential Eagle’s medium (α-MEM, Thermo Fisher Scientific, MA, USA) at 37 °C for 40 min (repeated for five times). After digestion, the supernatant was mixed and centrifuged to pellet cells. The cells were then maintained in α-MEM containing 10% fetal bovine serum (FBS, Thermo Fisher Scientific), 100 U/mL penicillin and 100 mg/mL streptomycin sulfate, at 37 °C with 5% CO₂. Next, the medium was replaced with α-MEM containing 1% bovine serum albumin (BSA, Thermo Fisher Scientific), and the cells were cultured for 16 h before preparing for subsequent experiments.

**ALP activity analyses**

Osteoblasts were evaluated by measuring ALP activity. ALP activity was measured using a commercial kit in accordance with manufacturer’s protocols (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

**Alizarin red staining**

The capability of mineralization of corresponding cells was assessed in 6-well plates using Alizarin red staining. The indicated cells were fixed with ice-cold 70% ethanol and stained with Alizarin red staining kit (Sigma-Aldrich, MO, USA) to detect the calcification according to the manufacturer’s protocols. ImageJ 1.8.0 software was applied to detect the percentages of positive areas, and then quantify the mineralized areas.

**Quantitative real-time PCR (qRT-PCR) assays**

Total RNA was extracted and purified by Trizol method. cDNA synthesis and quantitative real-time PCR (qRT-PCR) assays were carried out in accordance with manufacturer’s protocols (Takara, Tokyo, Japan). The designed primer sequences are as following:

**Specific primer sequences for qPCR**

| Gene  | Forward (5′–3′)         | Reverse (5′–3′)         |
|-------|-------------------------|-------------------------|
| PCNA  | ACTCGGATTTGCC TGGCATGG | TGACTACCGCTT TGTGGCTTTGG |
| Coll  | CACCTGGGCTGCT TACCTCGTCCT | GTTGGGCGTGAG CAGTTCAG |
| BGLAP | CACTCTCTGCCC TATTTGGC | CCCCTCTGCTTG GACACAAAA |
| OPN   | AGCAGCTTGGCC CAGACCTA | TAGGGCCGGAAT CTGTTCACTAC |
| GAPDH | ACCACAGTCTCAT GCCATCAC | TCCACACCTCTG TTGCTGTA |

After the reaction, the amplification curve and melting curve of qRT-PCR were confirmed. Each melting curve showed that a single peak was formed at 80–90 °C, and the peak shape was narrow. No primer dimer curve peak and other
heteropeaks were found. Based on the above observations, the specificity of each primer was qualified. Next, the relative quantitative assay of C(t) value was applied, that is, the C(t) value was calculated with the housekeeper gene (GAPDH) as the internal parameter, and then the relative ratio of the target gene content in the experimental sample and control sample was calculated with the control sample as the reference.

**Western blotting assays**

Total cellular protein was extracted using RIPA buffer (Beyotime, Jiangsu, China) and quantified using BCA protein assay kit (Beyotime, Jiangsu, China). The proteins were loaded and electrophoresed separately through a 15% SDS-PAGE gel. The separated proteins were subsequently transferred to the polyvinylidene fluoride membranes (PVDF) membrane and incubated with primary antibodies (rabbit anti-LC3, BCL2, Beclin1 and GAPDH; Cell Signaling Technology, Boston, USA) at 4 °C overnight. After washing, the membrane was incubated with the secondary antibody at room temperature for 60 min. The immunoreactive bands were visualized using an ECL kit (Millipore, MA, USA) and were quantified using a Chemi-Doc image analyser (Bio-Rad).

**Lentiviral transduction**

Recombinant lentiviruses encoding the wild-type BCL2 or shRNA against Beclin1 were constructed by homologous recombination between the expression vector (pEX-Puro-Lv105) and cDNA/shRNA in 293 cells using the lentivirus construction kit in accordance with manufacturer’s protocols. The same method was used to construct and package the corresponding control vector. After 2 days, supernatants were harvested, and primary osteoblasts were incubated in medium containing lentiviruses and 5 μg/mL polybrene at a multiplicity of infection (MOI) of 40 for 2 days. The infected cells were selected using puromycin (10 μg/mL). The overexpression efficiency of viral gene was detected using qPCR analysis.

**Coimmunoprecipitation (Co-IP) assays**

The total protein was extracted by RIPA Lysis and Extraction Buffer (Thermol Fisher Scientific). Subsequently, we rinsed the beads with 100 μL iced buffer, added 100 μL antibody-binding buffer to revolve the antibody and magnetic beads for 30 min, and then rinsed the beads three times using 200 μL buffer for 5 min each time. Cell lysates and antibody-bound magnetic beads were incubated for 1 h at room temperature and washed using 200 μL buffer for 5 min each time. 20 μL eluent was used to rinse the beads once and the supernatant was removed. The cell lysates were extracted for Co-IP with anti-BCL2 antibody (Cell Signaling Technology), and subsequently, precipitates were examined using Western blotting with anti-Beclin1 antibody.

**Transmission electron microscopy (TEM) analyses**

After treatment with the indicated interventions, the preparation of cell sections, staining, and TEM assays were performed according to manufacturer’s protocols (Servicebio, Wuhan, China). The cell ultrastructures were observed under TEM (Hitachi, Tokyo, Japan).

**Statistical analysis**

Data are expressed as mean ± SEM. Statistical analyses were performed using SPSS19.0. For comparisons, one-way ANOVA or Student’s t-test was performed. Tukey test was used for Post-Hoc Multiple Comparisons of one-way ANOVA. Differences were considered significant at a threshold of P < 0.05.

**Results**

**Lactoferrin promoted the autophagy activity of osteoblasts**

We first observed the effect of lactoferrin on the autophagy activity of primary osteoblasts. As shown in Fig. 1A–C, lactoferrin increased ALP activity and alizarin red-positive areas of osteoblasts in a concentration-dependent manner, which showed the effectiveness of lactoferrin in this experimental system. It was detected that lactoferrin can enhance the LC3 transformation rate (the ratio of LC3II to LC3I) in the presence or absence of lysosomal protease inhibitor (E64D + Pepstain A) (Fig. 1D). It could be observed that the administration of lysosomal protease inhibitor upregulated the LC3 transformation of osteoblasts (Fig. 1D), which indicated that autophagy flux is unobstructed and our experimental system is reliable. In addition, lactoferrin increased the number of autophagosomes in osteoblasts (Fig. 1E, F). These results supported that lactoferrin could stimulate the autophagy activity of osteoblasts.

**Lactoferrin inhibited the BCL2 protein expression in osteoblasts**

Then, we examined the regulatory ability of lactoferrin on BCL2 protein expression in osteoblasts. It was observed that under the intervention of different concentrations of lactoferrin, BCL2 protein expression decreased in a concentration-dependent manner, while Beclin1 protein level increased in a
concentration-dependent manner (Fig. 2A). However, BCL2 mRNA expression was not affected by lactoferrin administration. These results suggested that lactoferrin can inhibit BCL2 protein production in osteoblasts (Fig. 2B). As shown in Fig. 2C, the BCL2 protein level of osteoblasts inhibited by lactoferrin is reversed by chloroquine administration, supporting that autophagy plays an important role in the BCL2 protein expression in osteoblasts regulated by lactoferrin. Consistently, it was found that lactoferrin inhibited the coimmunoprecipitation level of BCL2 and Beclin1 in osteoblasts (Fig. 2D), indicating the dissociation effect of lactoferrin on BCL2-Beclin1 complex in osteoblasts.

BCL2 overexpression reversed lactoferrin-promoted osteoblast autophagy

We documented that lactoferrin could stimulate the autophagy activity and inhibit BCL2 protein expression in osteoblasts. We further investigated the role of BCL2 in lactoferrin-regulated osteoblast autophagy. First, we upregulated BCL2 expression level in osteoblasts by using Gene-overexpressing technology. The overexpression efficiency of BCL2 was verified via qPCR (2.7-fold) and Western blotting (1.81-fold) (Fig. 3A, B). As shown in Fig. 3C, lactoferrin enhanced the LC3 transformation in osteoblasts, which was reversed by BCL2 overexpression. In addition, the osteogenic parameters enhanced by lactoferrin (ALP activity and mRNA expression of PCNA, Coll, BGLAP and OPN) were recovered by BCL2 overexpression (Fig. 3D–H). These results indicated the important role of BCL2 in lactoferrin-regulated osteoblast formation and autophagy.

Beclin1 inhibition reversed lactoferrin-promoted osteoblast autophagy

We documented that lactoferrin could promote Beclin1 protein expression and dissociate BCL2-Beclin1 complex in osteoblast. We further explored the significance of Beclin1 in lactoferrin-regulated osteoblast autophagy. First, we downregulated Beclin1 expression level in osteoblasts by Gene-silencing technology. The silencing efficiency of Beclin1 was verified via qPCR (0.22-fold) and Western blotting (0.49-fold) (Fig. 4A, B). As shown in Fig. 4C, the LC3 transformation in osteoblasts enhanced by lactoferrin was recovered by Beclin1 silencing. In addition, the osteogenic parameters enhanced by lactoferrin (ALP activity
and mRNA expression of PCNA, Col1, BGLAP and OPN) were reversed by Beclin1 silencing (Fig. 4D–H). These results suggested the significance of Beclin1 in lactoferrin-regulated osteoblast formation and autophagy.

Discussion

Lactoferrin is an anti-inflammatory factor derived from milk, which can promote the osteogenesis and subsequent bone remodeling [2–13]. In addition, as an important autophagy regulator, lactoferrin can promote the autophagy activity of multiple cells [22–24]. Autophagy plays an indispensable role in the osteoblast formation [14–21], which provides us with an interesting scientific question, how lactoferrin affect the autophagy of osteoblasts. There is no effective evidence for the above problem. Our study is the first to reveal a scientific discovery: lactoferrin regulates the autophagy and formation of osteoblasts through BCL2-Beclin1 signaling.

First, we confirmed that lactoferrin not only directly promoted the growth of primary osteoblasts but also enhanced the autophagy activity of osteoblasts. Therefore, the direct effect of lactoferrin on osteoblast autophagy was proved for the first time, which acts in osteoblast formation promoted by lactoferrin. In addition, it was found that lactoferrin also downregulated BCL2 protein expression and the coimmunoprecipitation ability of BCL2 and Beclin1. It is well known that BCL2-Beclin1 complex plays a key role in autophagy activation [26–30]. This study is the first to elucidate that lactoferrin can promote Beclin1 into autophagy flux by reducing BCL2 protein level, thus activating autophagy. Remarkably, lactoferrin had no effect on BCL2 mRNA level. Furthermore, the application of chloroquine, an autophagy inhibitor, reversed the reduced BCL2 protein level by lactoferrin. Chloroquine, as a late autophagy inhibitor, can prevent the fusion of autophagosome and lysosome and the digestion of autolysosome on the substances contained. These results suggest that lactoferrin may promote the autophagic degradation
of BCL2 through autophagy activation. Accordingly, there may be a cycle in lactoferrin-regulated osteoblast formation, BCL2-autophagy-BCL2. Obviously, overexpression of BCL2 gene or knockdown of Beclin1 gene can recover the autophagy and formation of osteoblasts enhanced by lactoferrin, further identifying the significance of BCL2-Beclin1 signaling in the autophagy and formation of osteoblasts treated by lactoferrin. Lactoferrin has anti-osteoporotic effect, but this effect is extremely limited. Therefore, it is necessary to deepen the exploration regarding the mechanism of lactoferrin in osteogenesis, which is conducive to the purification of therapeutic components of lactoferrin in future. Based on the role of BCL2-Beclin1-autophagy activation pathway in lactoferrin-regulated osteoblast formation, the anti-osteoporotic effect of lactoferrin is expected to be improved from the corresponding scientific dimension in future. The above findings shed lights on optimizing the clinical value of lactoferrin in osteoporosis. The working model regarding our study is described in Fig. 5.

As an effective component of dairy products, lactoferrin is beneficial to bone formation and integrity. Our data elucidate the intrinsic mechanism underlying the direct effect of lactoferrin on osteoblast formation from the perspective of autophagy. The current results not only unmask a novel signal transduction pathway existing in lactoferrin-regulated osteoblast formation: BCL2-Beclin1-autophagy activation signaling, but also provide an important reference for the clinical application of lactoferrin in pathological bone loss.

Fig. 3 BCL2 overexpression reverses lactoferrin-promoted osteoblast autophagy. A mRNA level of BCL2 in osteoblasts infected with lentiviruses encoding BCL2-cDNA (LV-BCL2) or control viruses (LV-Cont) were detected using qRT-PCR assays. B The protein level of BCL2 in osteoblasts infected with lentiviruses encoding BCL2-cDNA (LV-BCL2) or control viruses (LV-Cont) were detected using Western blotting assays. C Following lentiviral transduction, the ratio of LC3II/I in osteoblasts treated with lactoferrin for 12 h was detected using Western blotting assays. D Following lentiviral transduction, ALP activity in osteoblasts treated with the same reagents as C for 5 days was measured using a commercial kit. E–H Following lentiviral transduction, mRNA levels of PCNA, Col1, BGLAP and OPN in osteoblasts treated as described in D were detected using qPCR assays. Data are presented as mean ± SEM from three independent experiments. *P < 0.05, ***P < 0.001.
Author's contributions DK, YL and SW conceived and designed the overall experiments; DK and SW carried out experiments, analyzed data, and prepared figures; XW assisted in experimental preparation and data analysis; DK and SW wrote the manuscript.

Funding This work was supported by Jiangmen Science and Technology Program in Basic and Theoretical Science Research (2020-159) and The Postdoctoral Science Foundation of China (2020M672731).

Fig. 4 Beclin1 inhibition reverses lactoferrin-promoted osteoblast autophagy. A mRNA level of BCL2 in osteoblasts infected with lentiviruses encoding Beclin1-shRNA (LV-sh-Beclin1) or control viruses (LV-sh-Cont) were detected using qRT-PCR assays. B The protein level of BCL2 in osteoblasts infected with lentiviruses encoding Beclin1-shRNA (LV-sh-Beclin1) or control viruses (LV-sh-Cont) were detected using Western blotting assays. C Following lentiviral transduction, the ratio of LC3II/I in osteoblasts treated with lactoferrin for 12 h was detected using Western blotting assays. D Following lentiviral transduction, ALP activity in osteoblasts treated with the same reagents as C for 5 days was measured using a commercial kit. E–H Following lentiviral transduction, mRNA levels of PCNA, Col1, BGLAP and OPN in osteoblasts treated as described in D were detected using qPCR assays. Data are presented as mean±SEM from three independent experiments. *P<0.05, ***P<0.001. LF lactoferrin

Declarations

Conflict of interest None of the authors disclosed potential conflicts of interest.

Research involving human and/or animal participants This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Włodarski K (2009) Lactoferrin—a promising bone-growth promoting milk-derived glycoprotein. Chir Narzadow Ruchu Ortop Pol 74(257–259):322–323
2. Hou JM, Xue Y, Lin QM (2012) Bovine lactoferrin improves bone mass and microstructure in ovariectomized rats via OPG/RANKL/ RANK pathway. Acta Pharmacol Sin 33:1277–1284. https://doi. org/10.1038/aps.2012.83
3. Guo HY, Jiang L, Ibrahim SA, Zhang L, Zhang H, Zhang M, Ren FZ (2009) Orally administered lactoferrin preserves bone mass and microarchitecture in ovariectomized rats. J Nutr 139:958–964. https://doi.org/10.1093/jn/108.10.100586
4. Shi P, Fan F, Chen H, Xu Z, Cheng S, Lu W, Du M (2020) A bovine lactoferrin-derived peptide induced osteogenesis via
regulation of osteoblast proliferation and differentiation. J Dairy Sci 103:3950–3960. https://doi.org/10.3168/jds.2019-17425
5. Chen XW, Li YH, Zhang MJ, Chen Z, Ke DS, Xue Y, Hou JM (2019) Lactoferrin ameliorates aging-suppressed osteogenesis via IGF1 signaling. J Mol Endocrinol 63:63–75. https://doi.org/10.1530/JME-19-0003
6. Li Y, Wang J, Ren F, Zhang W, Zhang H, Zhao L, Zhang M, Cui W, Wang X, Guo H (2018) Lactoferrin promotes osteogenesis through TGF-β receptor II binding in osteoblasts and activation of canonical TGF-β signaling in MC3T3-E1 cells and C57BL/6J mice. J Nutr 148:1285–1292. https://doi.org/10.1093/jn/nxy097
7. Zhang JL, Han X, Shan YJ, Zhang LW, Du M, Liu M, Yi HX, Ma Y (2018) Effect of bovine lactoferrin and human lactoferrin on the proliferative activity of the osteoblast cell line MC3T3-E1 in vitro. J Dairy Sci 101:1827–1833. https://doi.org/10.3168/jds.2017-13161
8. Huang L, Yang Z, Liu R, Xiao A, Zhou C, Yin X, Zou S, Chen J (2021) Lactoferrin promotes osteogenesis of MC3T3-E1 cells induced by mechanical strain in an extracellular signal-regulated kinase 1/2-dependent manner. Am J Orthod Dentofac Orthop 159:e113–e121. https://doi.org/10.1016/j.ajodo.2020.08.015
9. Xu D, Song W, Zhang J, Liu Y, Lu Y, Zhang X, Liu Q, Yuan T, Liu R (2021) Osteogenic effect of polydimethyl methacrylate bone cement with surface modification of lactoferrin. J Bioactive Biofilm 13:132–139. https://doi.org/10.1016/j.jbiofilm.2021.04.006
10. Liu M, Fan F, Shi P, Tu M, Yu C, Yu C, Du M (2018) Lactoferrin promotes MC3T3-E1 osteoblast cells proliferation via MAPK signaling pathways. Int J Biol Macromol 107:137–143. https://doi.org/10.1016/j.ijbiomac.2017.08.151
11. Li Y, Huang J, Wang J, Ma M, Lu Y, Wang R, Guo H (2021) Lactoferrin is a potential activator of the vitamin D receptor in its regulation of osteogenic activities in C57BL/6 mice and MC3T3-E1 cells. J Nutr 151:2105–2113. https://doi.org/10.1093/jn/nxab105
12. Zhang Y, Zhang ZN, Li N, Zhao LJ, Xue Y, Wu HJ, Hou JM (2020) Nbr1-regulated autophagy in lactoferrin-induced osteoblastic differentiation. Biosci Biotechnol Biochem 84:1191–1200. https://doi.org/10.1080/09116845.2020.1737505
13. Kim SE, Choi S, Hong JY, Shin KS, Kim TH, Park K, Lee SH (2019) Accelerated osteogenic differentiation of MC3T3-E1 cells by lactoferrin-conjugated nanodiamonds through enhanced anti-oxidant and anti-inflammatory effects. Nanomaterials (Basel) 9:10. https://doi.org/10.3390/nnano9050010
14. Weng YM, Ke CR, Kong JZ, Chen H, Hong JJ, Zhou DS (2018) The significant role of ATG5 in the maintenance of normal functions of MC3T3-E1 osteoblast. Eur Rev Med Pharmacol Sci 22:1224–1232. https://doi.org/10.26355/eurrev_201803_14462
15. Vidoni C, Ferraresi A, Secomandi E, Vallino L, Gardin C, Zavan B, Mortellaro C, Isidoro C (2019) Autophagy drives osteogenic differentiation of human gingival mesenchymal stem cells. Cell Commun Signal 17:98. https://doi.org/10.1186/s12964-019-0414-7
16. Kang C, Wei L, Song B, Chen L, Liu J, Deng B, Pan X, Shao L (2017) Involvement of autophagy in tantalum nanoparticle-induced osteoblast proliferation. Int J Nanomed 12:4323–4333. https://doi.org/10.2147/IJN.S136281
17. Lian WS, Ko JY, Chen YS, Ke HC, Wu SL, Kuo CW, Wang FS (2018) Chaperonin 60 sustains osteoblast autophagy and counteracts glucocorticoid aggravation of osteoporosis by chaperoning RPTOR. Cell Death Dis 9:938. https://doi.org/10.1038/s41419-019-0970-6
18. Yang YH, Li B, Zheng XF, Chen JW, Chen K, Jiang SD, Jiang LS (2014) Oxidative damage to osteoblasts can be alleviated by early autophagy through the endoplasmic reticulum stress pathway–implications for the treatment of osteoporosis. Free Radic Biol Med 77:10–20. https://doi.org/10.1016/j.freeradbiomed.2014.08.028
19. Huang Z, Wang Q, Zhang T, Fu Y, Wang W (2021) Hyper-activated platelet lysates prevent glucocorticoid-associated femoral head necrosis by regulating autophagy. Biomed Pharmacother 139:111711. https://doi.org/10.1016/j.biopha.2021.111711
20. Liu P, Cui Y, Liu M, Xiao B, Zhang J, Huang W, Zhang X, Song M, Li Y (2021) Protective effect of mitophagy against aluminum-induced MC3T3-E1 cells dysfunction. Chemosphere 282:131086. https://doi.org/10.1016/j.chemosphere.2021.131086
21. Wang N, Xu P, Wu R, Wang X, Wang Y, Shou D, Zhang Y (2021) Timosaponin BII improved osteoporosis caused by hyperglycemia through promoting autophagy of osteoblasts via suppressing the mTOR/NFκB signaling pathway. Free Radic Biol Med 171:112–123. https://doi.org/10.1016/j.freeradbiomed.2021.05.014
22. Huang L, Chen R, Liu L, Zhou Y, Chen Z (2021) Lactoferrin ameliorates pathological cardiac hypertrophy related to mitochondrial quality control in aged mice. Food Funct 12:7514–7526. https://doi.org/10.1039/d0fo03346d
23. Guo C, Xue H, Guo T, Zhang W, Xuan WQ, Ren YT, Wang D, Chen YH, Meng YH, Gao HL, Zhao P (2020) Recombinant human lactoferrin attenuates the progression of hepatosteatosis and hepatocellular death by regulating iron and lipid homeostasis in ob/ob mice. Food Funct 11:7183–7196. https://doi.org/10.1039/d0fo00910e
24. Hsu YH, Chiu JJ, Lin YF, Chen YJ, Lee YH, Chiu HW (2020) Lactoferrin contributes to a renoprotective effect in acute kidney injury and early renal fibrosis. Pharmaceutics 12:434. https://doi.org/10.3390/pharmaceutics12050434
25. Pattingre S, Tassa A, Xu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, Levine B (2005) Bcl-2 anti-apoptotic proteins inhibit Beclin1-dependent autophagy. Cell 122:927–939. https://doi.org/10.1016/j.cell.2005.07.002
26. Wei Y, Pattingre S, Sinha S, Bassik M, Levine B (2008) JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. Mol Cell 30:678–688. https://doi.org/10.1016/j.molcel.2008.06.001
27. Ke D, Li J, Wang Y, Fu X, Chen J, Wang F, Zhao D, Xue Y, Lan X, Hou J (2019) JNK1 regulates RANKL-induced osteoclastogenesis via activation of a novel Bcl-2-Beclin1-autophagy pathway. FASEB J 33:11082–11093. https://doi.org/10.1096/fj.201802597R
28. Duang Y, Song R, Li N, Sun K, Shi F, Liu H, Shen F, Jiang S, Zhang L, Jin Y (2020) Silica dust exposure induces autophagy in alveolar macrophages through switching Beclin1 affinity from Bcl-2 to PIK3C3. Environ Toxicol 35:758–767. https://doi.org/10.1002/tox.22910
29. Guo QQ, Wang SS, Zhang SS et al (2020) ATM-CHK2-Beclin 1 axis promotes autophagy to maintain ROS homeostasis under oxidative stress. EMBO J 39:e103111. https://doi.org/10.15252/embj.2019103111
30. Li S, Lin Z, Zheng W, Zheng L, Chen X, Yan Z, Cheng Z, Yan H, Zheng C, Guo P (2019) IL-17A inhibits autophagic activity of HCC cells by inhibiting the degradation of Bcl-2. Biochem Biophys Res Commun 509:194–200. https://doi.org/10.1016/j.bbrc.2018.12.103

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.