The Role of Galectin-3 in 1α,25(OH)₂D₃-Regulated Osteoclast Formation from White Leghorn Chickens In Vitro

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Abstract: Bones play an important role in maintaining the level of calcium in blood. They provide support for soft tissues and hematopoiesis and undergo continuous renewal throughout life. In addition, vitamin D is involved in regulating bone and calcium homeostasis. Galectin-3 (Gal-3) is a β-galactoside-binding protein that can regulate bone cell differentiation and function. Here, we aimed to study the regulatory effects of Gal-3 on vitamin-D-regulated osteoclastogenesis and bone resorption in chicken. Gal-3 expression in bone marrow stromal cells (BMSCs) from 18-day-old chicken embryos was inhibited or overexpressed. BMSCs were then co-cultured with bone marrow monocytes/macrophages (BMMs) with or without addition of 1α,25(OH)₂D₃. The results showed that 1α,25(OH)₂D₃ upregulated the expression of Gal-3 mRNA and receptor activator of nuclear-factor κB ligand (RANKL) expression in BMSCs and promoted osteoclastogenesis, as shown by the upregulated expression of osteoclast (OC) markers (CtsK, CAIII, MMP-9, and TRAP) and increased bone resorption, a method for measuring the bone resorption area in vitro. Knockdown of Gal-3 by small-interfering RNA (siRNA) in BMSCs downregulated the expression of RANKL mRNA and attenuated the effects of 1α,25(OH)₂D₃ on osteoclastogenesis and bone resorption. Conversely, overexpression of Gal-3 in BMSCs enhanced the effects of osteoclastogenesis and bone resorption by increasing the expression of RANKL mRNA. These results demonstrated that Gal-3 mediates the differentiation and bone resorption of osteoclasts regulated by 1α,25(OH)₂D₃.

Keywords: galectin-3 (Gal-3); bone marrow stromal cells (BMSCs); bone marrow monocytes/macrophages (BMMs); osteoclasts (OCs); 1α,25-dihydroxyvitamin D₃ (1α,25(OH)₂D₃)

1. Introduction

Bone homeostasis is achieved by osteogenesis and bone resorption, which are regulated by osteoblasts (OBs) and osteoclasts (OCs), respectively [1,2]. OCs are specifically responsible for physiological and pathological bone resorption. Excessive increase or decrease in their quantity and activity is detrimental to bone and calcium (Ca) homeostasis [3,4]. Vitamin D is a sterol derivative and has long been considered to promote osteogenesis by promoting the intestinal transport of Ca and osteolysis, ensuring the stability of serum calcium homeostasis. Previous studies have shown that 1α,25-dihydroxyvitamin D₃ (1α,25(OH)₂D₃) can upregulate receptor activator of nuclear-factor κB ligand (RANKL) expression in OBs and bone narrow...
stromal cells (BMSCs) in mammals, indirectly inducing bone marrow monocytes/macrophages (BMMs) to differentiate into OCs for bone resorption [5–7].

Galectin-3 (Gal-3) is a member of the β-galectin family and presents in the nucleus. Gal-3 can regulate cell migration, adhesion, apoptosis, and gene expression [8,9]. Studies have shown that Gal-3 is expressed in chondrocytes, OBs, BMSCs, and OCs and regulates osteocyte differentiation and function [10,11]. In Gal-3 (Gal-3−/−) knockout mice, OB and OC differentiation and bone resorption were impaired [12]. It has also been confirmed that Gal-3 could overlap with the transcription factor Runx2, which regulates OB differentiation [13]. Furthermore, Gal-3 can regulate bone marrow mesenchymal stem cell migration through RhoA-GTP signaling and may be a potential target for treating bone reconstruction-related diseases [14]. Aubin et al. found that 1α,25(OH)2D3 promoted Gal-3 expression in a rat OB-derived sarcoma cell line, ROS 17/2.8 [15]. Simon et al. reported that knockout Gal-3 in OBs displayed higher osteoclastogenesis, independently of the RANKL signaling pathway [16]. However, the role of Gal-3 in 1α,25(OH)2D3-regulated osteoclastogenesis and bone resorption and whether it affects vitamin D regulation of osteoclastogenesis in poultry (chicken) is unclear.

Chicken bones have been used in bone development and bone injury studies for a long time as they are similar to those of humans and other vertebrates, and vitamin D has been found to regulate bone development in chickens [17–19]. Our previous study showed that 1α,25(OH)2D3 promoted osteoclastogenesis in a chicken BMSC–BMM co-culture system in a dose-dependent manner, with 10−9 mol/L having the most significant effect [20]. In addition, 10−8 mol/L 1α,25(OH)2D3 was used in the current study to further examine the effects of vitamin D on Gal-3 expression in BMSCs, BMMs, and BMSC–BMM co-culture. Small-interfering RNA (siRNA) and gene overexpression were used to knockdown or overexpress Gal-3 to observe the effects of 1α,25(OH)2D3 on osteoclastogenesis, bone resorption, and RANKL signaling and to examine whether Gal-3 affects 1α,25(OH)2D3 regulation of osteoclastogenesis through RANKL signaling. These results will provide a foundation for studies on vitamin D regulation of bone and calcium homeostasis in poultry.

2. Materials and Methods

2.1. Animals

The white leghorn chicken embryos used here were fertilized SPF-grade eggs from Single Comb White Leghorn (Yigida Biotechnology, Jining, China). The SPF-grade eggs were incubated at 37 °C and 60% humidity until they were 18 days old. The animal use was approved by the Animal Care and Use Committee of Yangzhou University (SYXX [Su] 2016-0020).

2.2. Isolation of BMSCs and BMMs

Tibias and femurs were separated, and bone marrow cells were filtered with a 200-mesh sieve and then centrifuged at 1200 r/min for 5 min. The cells were subsequently resuspended in α-MEM (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% FBS (EallBio, Beijing, China) and then incubated in an incubator (5% CO2, 37 °C). After 2 days, the BMSCs were adherent and the BMMs were non-adherent.

2.3. Transfection

The sequence of Gal-3 siRNA was designed as 5′-AGAGAACAGCTCCTAGATT-3′, and the sequences of negative control (NC) siRNA were designed as 5′-GCGCTCTAGAAAGCCTATGC-3′ (Ribobio, Guangzhou, China).

BMSCs were seeded in cell culture plates (Corning, New York, NY, USA) or bone resorption cultural plates (Sigma-Aldrich, St. Louis, MO, USA). After 12 h, the BMSCs were transfected with siRNAs. Transfection was performed using Lipofectamine™ 2000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions, as previously reported [21].
2.4. Overexpression of Gal-3 Plasmids

Gal-3 homologous recombination was performed with PEXP-RB-MAM-EGFP transient vector (RiboBio, Guangzhou, China). The gene EGFP-Gal-3 was used for cell transfection, and the empty carrier control was named EGFP. Transfection was performed using Lipofectamine™ 3000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions, as previously reported [21].

2.5. Co-Culture of BMSCs and BMMs

BMSCs were seeded into cell cultural plates or bone resorption cultural plates at a ratio of 1:100 (BMSCs:BMMs). Cells were then treated with 10⁻⁸ mol/L 1α,25(OH)₂D₃ (Sigma-Aldrich, USA) for 5 d (control group without 1α,25(OH)₂D₃). The medium was changed every 2 days. At the end of the incubation period, the medium was decanted from the cell cultures. BMSCs were transfected with NC siRNA and Gal-3 siRNA for 10 h. BMMs were seeded into these transfected BMSC cultures at a ratio of 1:100 (BMSCs:BMMs).

At the end of the incubation period, the medium was decanted from the cell cultures. BMSCs were transfected with EGFP plasmid and EGFP-Gal-3 for 10 h. BMMs were transferred into BMSCs at a ratio of 100:1 (BMMs:BMSCs). Then, the medium was changed to α-MEM (containing 10% FBS) for 5 d. The medium was changed every 2 days.

2.6. Identification of Osteoclastogenesis

At the end of the incubation period, the medium was decanted from the cell cultures. Cells were fixed with 4% paraformaldehyde for 10 min (New Cell & Molecular Biotech Co., Ltd., Hangzhou, China). Tartrate-resistant Acid Phosphatase (TRAP) staining solution was added according to the manufacturer’s instructions (Sigma-Aldrich, St. Louis, MO, USA), as previously described [22].

2.7. Identification of Bone Resorption

At the end of the incubation period, the medium was decanted from the cell cultures. PBS was used to repeatedly wash the plates, and photographs were subsequently taken under an inverted microscope. Image-Pro Plus software was used for calculation of the area of the bone resorption pits.

2.8. qRT-PCR

RNA was extracted using Trizol (Thermo Fisher Scientific, Waltham, MA, USA) and qRT-PCR following the manufacturer’s instructions, as previously described [23]. The expression of the targeting genes cathepsin K (CtsK), TRAP, matrix metalloproteinase-9 (MMP-9), carbonic anhydrase II (CaII), RANKL, osteoprotegrin (OPG), and Gal-3 mRNA was measured. GAPDH was used as an internal reference. All primers used are shown in Table 1.

| Name                | Gene Number | Length (bp) | Forward Primer (5' to 3') | Reverse Primer (5' to 3') |
|---------------------|-------------|-------------|---------------------------|---------------------------|
| CtsK (chicken)      | 395818      | 161         | CATCATGGACGGAGCCATGC      | TTTGTCCTTCTTGCCGTG        |
| TRAP (chicken)      | 107057619   | 110         | CGGTTCTTCTATGGGTCTCT      | GGTACAGAATCATCCTCCGTTGG   |
| MMP-9 (chicken)     | 395387      | 182         | CTTCGCCCCAGACAGGTTT       | AGCCAGACCATAGAGTCTACT     |
| CaII (chicken)      | 396257      | 188         | GCCGGTGAAGTACGAGTCAA      | GCTGTGATCCCTGGTTGAA       |
| RANKL (chicken)     | 428867      | 116         | GCCCACTTCTTGGAAAACGC      | TACAAAGTGGGCTGTTGAA       |
| OPG (chicken)       | 378803      | 90          | CTGCAGCCTTGGCTCTGG        | GATGTCGGGGTGCTAAATGG      |
| FGF23 (chicken)     | 373917      | 112         | GCCATATCTCTGGAGGACC       | GGTTATGAGCAGTGGAGCCA      |
| GAPDH (chicken)     | 374193      | 137         | GCCCAAACATCATCCCAG        | CGCCAGTCAGGTCACAGA        |
2.9. ELISA

The supernatants of cells were collected, and the levels of CtsK, MMP-9, RANKL, and OPG were measured using chicken ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd, Shanghai, China) according to the manufacturer’s instructions. The OD value was measured at 450 nm within 15 min. Protein content was calculated based on the OD.

2.10. Statistical Analysis

Results are expressed as the mean ± SD from at least three independent experiments. Significance was calculated by one-way analysis of variance (ANOVA) using SPSS 19.0 software. p-values lower than 0.05 were considered statistically significant.

3. Results

3.1. 1α,25(OH)2D3 Promoted Osteoclastogenesis and Bone Resorption

BMSCs were co-cultured with BMMs for 5 d with or without the addition of 10−8 mol/L 1α,25(OH)2D3. As shown in Figure 1A,B, 10−8 mol/L 1α,25(OH)2D3 significantly (p < 0.01) promoted osteoclastogenesis, characterized by multinucleation and the wine-colored cytoplasm. Similarly, 10−8 mol/L 1α,25(OH)2D3 significantly (p < 0.01) upregulated the expression of CtsK, TRAP, MMP-9, and CAII mRNA (Figure 1C) and significantly (p < 0.05) increased the protein levels of CtsK and MMP-9 in the medium supernatant (Figure 1D).

In addition, the area of bone resorption pits was significantly (p < 0.01) increased in the 10−8 mol/L 1α,25(OH)2D3 group (Figure 1E,F). These results show that 10−8 mol/L 1α,25(OH)2D3 promotes osteoclastogenesis and bone resorption in the chicken BMSC–BMM co-culture system.

![Figure 1](image.png)

Figure 1. Culture with 10−8 mol/L 1α,25(OH)2D3 promotes osteoclastogenesis and bone resorption in the chicken BMSC–BMM co-culture system. (A,B) BMSCs and BMMs were co-cultured on 6-well plates with or without (Con) addition of 10−8 mol/L 1α,25(OH)2D3. Cells were treated for 5 d, and 10 fields were randomly selected. The number of TRAP-positive multinucleated OCs (black arrows) was counted. Bar = 100 μm. (C,D) BMSCs and BMMs were co-cultured on 6-well plates with or without (Con) addition of 10−8 mol/L 1α,25(OH)2D3. Cells were treated for 5 d, and qRT-PCR was used to quantitate the mRNA expression of OC marker genes (CtsK, TRAP, MMP-9, and CAII). Chicken ELISA kits were used to measure CtsK and MMP-9 protein levels in the cell culture supernatant (n = 3 per group). (E,F) BMSCs alone, no cells, and BMSC–BMM were added to a 96-well bone resorption culture plate. After that, 10−8 mol/L 1α,25(OH)2D3 was added or not added (Con) to BMSC–BMM co-culture wells for 5 d. An inverted microscope was used to observe 10 randomly selected fields. Image-Pro Plus 6.0 software was used to analyze the area of bone resorption pits (red arrows). Bar = 50 μm. (Graph bars show mean ± SD. * p < 0.05 and ** p < 0.01 show significant difference compared with the control group).
3.2. 1α,25(OH)2D3 Promoted the Expression of Gal-3

Application of 10−8 mol/L 1α,25(OH)2D3 significantly (p < 0.05 or p < 0.01) upregulated the expression of Gal-3 mRNA in the BMSC–BMM co-culture system, BMSCs, and BMMs (Figure 2A–C). These results indicated that 10−8 mol/L 1α,25(OH)2D3 increased the Gal-3 expression.

![Image](image1.png)

**Figure 2.** Application of 10−8 mol/L 1α,25(OH)2D3 upregulates the expression of Gal-3 mRNA in the BMSC–BMM co-culture system and monocultures. (A) BMSCs and BMMs were co-cultured in 6-well plates with or without (Con) 1α,25(OH)2D3. Quantitative RT-PCR was used to quantitate the expression of Gal-3 mRNA. (B) BMSCs were seeded in 6-well plates with or without (Con) 1α,25(OH)2D3, and qRT-PCR was used to quantitate the expression of Gal-3 mRNA. (C) BMMs were seeded in 6-well plates with or without (Con) 1α,25(OH)2D3, and qRT-PCR was used to quantitate the expression of Gal-3 mRNA. (Graph bars show mean ± SD. * p < 0.05 and ** p < 0.01 show significant difference compared with the control group; n = 3 per group).

3.3. 1α,25(OH)2D3 Promoted RANKL Expression and Inhibited OPG Expression

Application of 10−8 mol/L 1α,25(OH)2D3 significantly (p < 0.01) inhibited the expression of OPG mRNA in BMSCs, but the expression of RANKL mRNA and the ratio of RANKL/OPG mRNA were significantly (p < 0.01) increased compared with the control group (Figure 3A). Similarly, 10−8 mol/L 1α,25(OH)2D3 significantly (p < 0.01) upregulated the protein levels of RANKL protein and the ratio of RANKL/OPG protein in the medium supernatant but downregulated the protein levels of OPG protein (Figure 3B). This shows that 10−8 mol/L 1α,25(OH)2D3 increased the RANKL/OPG ratio in BMSCs.

![Image](image2.png)

**Figure 3.** Application of 10−8 mol/L 1α,25(OH)2D3 upregulates the RANKL/OPG ratio in BMSCs. (A) BMSCs were seeded in 6-well plates with or without (Con) 1α,25(OH)2D3. After 5 d, qRT-PCR was used to quantitate the expression of RANKL and OPG mRNA, and the RANKL/OPG ratio was calculated. (B) BMSCs were seeded in 6-well plates with or without (Con) 1α,25(OH)2D3. After 5 d, the cell supernatant was collected, and ELISA kits were used to measure RANKL and OPG protein levels. (Graph bars show mean ± SD. * p < 0.05 and ** p < 0.01 show significant difference compared with the control group; n = 3 per group).
3.4. Knockdown of Gal-3 Inhibited Osteoclastogenesis

BMSCs were transfected with targeting of Gal-3 siRNA for 36, 72, and 120 h, and the expression of Gal-3 mRNA was significantly ($p < 0.01$) decreased in the si-Gal-3 group at each time point (Figure 4A).

As shown in Figure 4B–F, $10^8$ mol/L 1α,25(OH)2D3 significantly ($p < 0.01$ or $p < 0.05$) promoted osteoclastogenesis compared with the NC group; upregulated the expression of CtsK, TRAP, MMP-9, and CAII mRNA; and enhanced the activity of bone resorption. However, knockdown of Gal-3 suppressed the expression of OC markers mRNA and the activity of bone resorption under treatment with $10^8$ mol/L 1α,25(OH)2D3. These results show that $10^8$ mol/L 1α,25(OH)2D3 regulates osteoclastogenesis and bone resorption in BMSCs through the regulation of Gal-3.

**Figure 4.** Gal-3 knockdown in BMSCs decreases the promoting effects of $10^8$ mol/L 1α,25(OH)2D3 on osteoclastogenesis and bone resorption. (A) BMSCs were transfected with si-Gal-3 or NC siRNA for 10 h before being cultured in α-MEM for 36, 72, or 120 h. Quantitative RT-PCR was used to quantify the expression of Gal-3 mRNA ($n = 3$ per group). (B,C) After Gal-3 knockdown in BMSCs, BMSCs and BMMs were co-cultured on 6-well plates with or without addition of 1α,25(OH)2D3. Cells were cultured for 5 days. NC was the blank control. Ten fields were randomly selected. The number of TRAP-positive multinucleated OCs (black arrows) was counted. Bar = 200 μm. (D) After Gal-3 knockdown, BMSCs were co-cultured with BMMs with or without addition of 1α,25(OH)2D3. Cells were cultured for 5 d. NC was the blank control. Quantitative RT-PCR was used to quantify the mRNA expression of OC marker genes (CtsK, CAII, MMP-9, and TRAP) ($n = 3$ per group). (E,F) After BMSCs were seeded in 96-well bone resorption culture plates and Gal-3 was knocked down, BMSCs and BMMs were co-cultured with or without addition of 1α,25(OH)2D3. Cells were cultured for 5 days. NC was the blank control. An inverted microscope was used for observation of 10 randomly selected fields. Image-Pro Plus 6.0 software was used to analyze the area of bone resorption pits (red arrows). Bar = 100 μm. (Graph bars show mean ± SD. * $p < 0.05$ and ** $p < 0.01$ show significant difference compared with the NC control group. * $p < 0.05$ and ** $p < 0.01$ show significant difference compared with the 1α,25(OH)2D3-treated si-Gal-3 group).
3.5. EGFP-Gal-3 Promoted OC Differentiation

BMSCs were transfected with EGFP-Gal-3 or EGFP for 36, 72, and 120 h. Compared with the EGFP group, the expression of Gal-3 mRNA significantly \((p < 0.01)\) increased following treatment with EGFP-Gal-3 (Figure 5A). In addition, the amount of TRAP; the expression of \(\text{CtsK}, \text{TRAP}, \text{MMP-9},\) and \(\text{CAII}\) mRNA; and the area of bone resorption were significantly \((p < 0.01 \text{ or } p < 0.05)\) increased in the EGFP-Gal-3 group (Figure 5B–F). These results indicate that overexpression of Gal-3 in BMSCs could upregulate osteoclastogenesis and bone resorption.

![Figure 5. Gal-3 overexpression in BMSCs promotes osteoclastogenesis and bone resorption.](image)
3.6. Effects of Si-Gal-3 and EGFP-Gal-3 on RANKL Expression in BMSCs

As shown in Figure 6, si-Gal-3 significantly ($p < 0.01$) inhibited the expression of RANKL mRNA, but there was no significant ($p > 0.05$) difference in the expression of OPG mRNA and the ratio of RANKL/OPG. Conversely, overexpression of Gal-3 significantly ($p < 0.05$) upregulated the expression of RANKL mRNA and the ratio of RANKL/OPG but significantly inhibited OPG expression ($p < 0.01$) (Figure 6B). These results show that Gal-3 could regulate RANKL expression in BMSCs.

![Figure 6](image.png)

**Figure 6.** Gal-3 can regulate RANKL signals. (A) BMSCs were transfected with si-Gal-3 or NC siRNA for 10 h before being cultured in normal α-MEM for 120 h. Quantitative RT-PCR was used to measure RANKL and OPG mRNA levels to analyze the RANKL/OPG ratio ($n = 3$ per group). (B) BMSCs were transfected with EGFP-Gal-3 or EGFP empty plasmid for 10 h before being cultured in normal α-MEM for 120 h. Quantitative RT-PCR was used to measure RANKL and OPG mRNA levels to analyze the RANKL/OPG ratio ($n = 3$ per group). (Graph bars show mean ± SD. * $p < 0.05$ and ** $p < 0.01$ show significant difference compared with the control group).

4. Discussion

Bone homeostasis depends on bone resorption in mammals and poultry; otherwise, it will lead to bone metabolism disorders. BMSCs are the progenitors of various cells (e.g., chondrocytes, adipocytes, and osteoblasts) which are recruited for microenvironmental stimulation of the bone surface, regulating osteoclastogenesis directly or indirectly [2,24]. BMSCs can secrete cytokines (e.g., RANKL and OPG) to regulate osteoclastogenesis [25–27]. In addition, activation of vitamin D promotes RANKL expression in BMSCs and OBs in vitro, regulating osteoclastogenesis [6,7,28]. In this study, treatment with $10^8$ mol/L $1\alpha,25$(OH)$_2$D$_3$ promoted the expression of RANKL and osteoclastogenesis.

Gal-3 is expressed at different locations in cells, including the nucleus, extracellularly, and in the cell membrane. Ortega et al. showed that Gal-3, an in vitro substrate of MMP-9, accumulates in late hypertrophic chondrocytes and the expanded hypertrophic cartilage zone. Furthermore, treatment with full-length Gal-3, but not that cleaved by MMP-9, led to expansion of the hypertrophic zone and inhibited osteoclast recruitment [29]. However, Nakajima et al. showed that Gal-3 cleavage in the bone tumor microenvironment (TME) of breast and prostate cancers delays osteoclastogenesis by targeting the carbohydrate recognition domain (CRD) of Gal-3 [30]. Next, we aimed to observe the role of Gal-3 in osteoclastogenesis in BMSCs and BMM co-culture system treated by $1\alpha,25$(OH)$_2$D$_3$. We found that application of $10^8$ mol/L $1\alpha,25$(OH)$_2$D$_3$ enhanced the expression of Gal-3 in BMSCs. A previous study reported that $1\alpha,25$(OH)$_2$D$_3$ upregulated the expression of Gal-3 in OBs [15]. In addition, knockout of Gal-3 in OBs and/or BMMs in mice caused
osteoclastogenesis, indicating that Gal-3 is involved in the differentiation of osteoclasts [16]. Our research found that knockdown of Gal-3 in BMSCs co-cultured with BMMs could suppress osteoclastogenesis and bone resorption. However, overexpression of Gal-3 could reverse it. In this study, we did not investigate the role of Gal-3 in regulating RANK in monocyte/macrophage since: (1) our study focused on the role of Gal-3 in regulating RANKL expression and osteoclastogenesis; (2) RANKL is expressed at very low levels in BMM; (3) 1α,25(OH)2D3 increased Gal-3 expression only weakly in BMM (2-fold) but dramatically in BMSC (>10-fold). In addition, the transfection efficiency is extremely low in macrophages, and it is technically challenging to conduct experiments in macrophages.

Next, the regulatory role of Gal-3 in the expression of RANKL was demonstrated in BMSCs. The results confirmed that the expression of RANKL mRNA was downregulated by knockdown of Gal-3, and the expression of RANKL mRNA and the ratio of RANKL/OPG were upregulated by overexpression of Gal-3. The current results are different from those of Simon et al., who reported that inhibition of osteoclastogenesis was regulated by Gal-3 in mice independently of the RANKL/OPG axis. This might be related to differences in species variation or the different degrees of knockdown or overexpression of Gal-3 used.

Our present study shows that 1α,25-(OH)2D3 increased the levels of Gal-3 and RANKL mRNA and promoted osteoclast differentiation and activation. Gal-3 knockdown led to decreased RANKL mRNA expression and blocked the effect of 1α,25-(OH)2D3 on osteoclast differentiation. Gal-3 overexpression increased RANKL mRNA levels. It is well documented that Gal-3 present in the nucleus and can bind several transcription factors and enhance their DNA-binding activity [31]. We propose that 1α,25(OH)2D3-induced Gal-3 expression affects osteoclast differentiation through RANKL signaling in chicken BMSC (Figure 7). However, the mechanisms by which 1α,25(OH)2D3 regulates Gal-3 expression and Gal-3 regulates RANKL expression need to be further investigated in detail.

Figure 7. Schematic diagram showing the mechanism of Gal-3 in 1α,25(OH)2D3-regulated differentiation of chicken osteoclasts. Application of 10−8 mol/L 1α,25(OH)2D3 upregulates Gal-3 expression in BMSC and regulates osteoclast differentiation in the chicken through RANKL signaling.
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Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors.

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