Construction of adenovirus vector expressing duck sclerostin and its induction effect on myogenic proliferation and differentiation in vitro

Hehe Liu · Yanying Li · Qian Xu · Jianmei Wang · Chunchun Han · Lili Bai · Liang Li

Received: 4 August 2021 / Accepted: 17 January 2022 / Published online: 6 February 2022
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Abstract

Background Bones and muscles originated together from the mesoderm during embryogenesis, and they can influence each other through mechanical stimulations and chemical signals. The sclerostin (SOST) is secreted from mature osteocytes. Here, we used a bird model to illustrate the potential roles of SOST on duck myoblasts to verify the hypothesis that SOST might play functions in coordinating the development of bones and muscles.

Methods and results Firstly, a recombinant adenovirus vector carrying duck SOST was constructed. Then, the adenovirus-mediated duck SOST was transfected into duck myoblasts. The results revealed by CCK-8 showed that the cell proliferation of myoblasts was inhibited after 12 h, 36 h, and 48 h treatment by transfection of SOST. The labeling rates of EdU positive cells in the Ad-duSOST group were significantly lower than the Ad-NC group (P < 0.05). However, the flow cytometry showed that the cells’ G0/G1 phase number was not significantly different. Furthermore, the immunofluorescence results showed that the formation of myotubes was inhibited. Subsequent transcriptome revealed that, under the ectopic expression of SOST, the genes related to Cytokine-cytokine receptor interaction, muscle development (regulation of action cytoskeleton, Wnt signaling pathway), and intercellular regulation were changed. Six of the top 20 DEGs were related to morphogenesis.

Conclusions Our studies demonstrated that the SOST played critical roles in myoblasts differentiation by mediating the crosstalk among several pathways and transcription factors related to cell differentiation. Our data provided cellular evidence supporting the combined functions of SOST in coordinating bone and muscle co-development.

Keywords Sclerostin · Duck · Myoblast · Differentiation · Recombinant adenovirus vector

Introduction

Vertebrates shared a common mesodermal origin as an intrinsic connection between bone and muscle during embryogenesis. The proximity in anatomical position, between bone and muscle, is more beneficial to their communication through mechanical and biochemical signals [1]. The bone provides an attachment surface for skeletal muscles, and muscle affects the structure of bone. Simultaneously, they could affect each other by transmitting biochemical molecules [2]. In humans, bones and muscles tend to degenerate together during aging or disease conditions, as patients with osteoporosis are more likely to get symptoms of muscle atrophy. Moreover, a sign of bone loss is often accompanied by muscular dystrophy in long-term bedridden patients [3].

Karsenty suggested that the paracrine pathway is the core link between bones and muscles. As the largest endocrine organs in vertebrates, bones and muscles can secrete various cytokines and growth factors, participating in the perception and transmission of signals, such as mechanical stretch stimuli and changes in nutritional levels [4]. The secreted proteins from bones, such as osteocalcin [5], Sclerostin (SOST) [6], and BMPs [7], can enter muscle tissues and eventually affect myofibers’ differentiation and protein metabolism through a paracrine pathway.

The SOST, secreted from the mature osteocytes, can mainly affect the function of osteoblasts and osteoclasts [8]. It could competitively bind to the co-receptor LRP5 of the Wnt/β-catenin signal pathway to inhibit osteoblast differentiation [9]. In vitro, the proliferation and differentiation of
osteoblasts were inhibited by the addition of SOST to the cell growth medium; however, they stimulated the process of apoptosis [10, 11]. Additionally, the serum level of SOST can be increased and decreased with the prolonged bed-rest time and higher muscle load, respectively [12]. The differentiation of C2C12 was promoted by adding the MLO-Y4 conditioned medium (CM) and then was inhibited by adding SOST [13]. These studies suggested that SOST might play dual roles in affecting the development of both bones and muscles. Our previous study demonstrated that the ossification process of the sternum in duck would affect pectoral muscle development. Moreover, in another experiment, we found that SOST was expressed differently between ducks’ calcified and un-calcified sternum. Therefore, it was hypothesized that the SOST might play combined roles in coordinating the co-development of bones and muscles, and the present study was designed to explore the potential effect of SOST on duck primary myoblasts, as well as provide evidence supporting the dual roles of SOST on both bones and muscles. The present study data might be used to illustrate the underlying mechanism of muscle and bone development-related diseases of humans.

Materials and methods

Construction of duck SOST recombinant adenovirus vector

After predicting the restriction enzyme sites (http://nc2.neb.com/NEBcutter2/) of duck SOST coding domain sequence (CDS, XM_005026106), the NEBuilder (http://nebuilder.neb.com/) was used to design primers with overlapping sequences of pAdTrack-CMV at both ends (Table S1). The entire CDS of duck SOST was amplified by PCR. After sequencing, the amplified PCR products of SOST and pAdTrack-CMV plasmid, linearized with XhoI and KpnI (Takara, China), were ligated with homologous recombinase (Biodragon Immunotechnologies Co., Ltd, China). Then, the recombinant plasmids were confirmed by sequencing and digestion. The recombinant plasmids were named pAdTrack-CMV-duSOST. The pAdTrack-CMV-duSOST linearized with PmeI and the adenoviral backbone plasmid (pAdEasy-1) were ligated with homologous recombinant in E. coli BJ5183 (Miaoling Bioscience & Technology Co., Ltd, China). The second recombinant plasmid was identified by PacI (NEB, China) restriction enzyme digestion and sequenced, which showed that the vector was successfully constructed and named pAd-duSOST (Fig. 1a). Finally, the second recombinant plasmid was subsequently used to generate recombinant adenoviruses in human embryonic kidney cells (HEK293; Kunming Institute of Zoology, China). The specific steps of adenovirus packaging in HEK293 cells are provided in the supplementary files. After collecting the adenovirus, it was continuously amplified in HEK293 for three generations and found that the adenovirus had stable infection abilities and was named Ad-duSOST.

The titer determination of recombinant adenovirus

According to Luo’s article, the titer of adenovirus was measured [14]. The HEK-293 cells were inoculated at 1 × 10⁴ cells/well in a 96-well plate and 90 μL/well complete culture medium containing 10% FBS after cell culture for 24 h. Then, 10 μL virus was added into the first well, and tenfold dilution of the previous virus-containing solution was performed before the virus was added into the next well (12 wells in each group, altogether three groups). After 48 h, an inverted fluorescence microscope (OLYMPUS IX73, Japan) was used to observe the fluorescence-positive cells in each well. The virus titer was calculated according to the following equation; virus titer (U/mL) = m × 10ⁿ⁺¹, where n is the serial number of the reference well, and m is the number of positive cells.

Duck myoblast isolation and culture

The fertilizer eggs of ducks were collected from the Water-fowl Breeding Farm of Sichuan Agricultural University. The eggs were kept incubating for 13 days under the same hatching conditions. The isolation and cell culture of duck myoblasts were according to the guidelines of our previous studies [15]. The primary myoblasts of ducks were cultured in a complete growth medium containing DMEM/F-12 (HyClone, China), 10% FBS, and 1% penicillin/streptomycin, and then were incubated in an incubator with 5% CO₂ at 37 °C. All animal experiments were allowed by the Animal Ethics Committee of Sichuan Agricultural University.

Multiplicity of infection (MOI) determination

The duck primary myoblasts were inoculated at 1 × 10⁵ cells/well in a 6-well plate, growing myoblasts (70–80% confluency) were infected with adenovirus solution of 30, 40, 50, 60, and 70 multiplicity of infection (MOI), respectively. The cells were collected after 48 h of infection, and the mRNA expression of the SOST gene in the cells was measured by real-time PCR.

Transfection of pAd-duSOST to myoblasts

The duck primary myoblasts were inoculated at 1 × 10⁵ cells/well in a 6-well plate and cultured in a complete medium (DMEM/F12 + 10% FBS + 100U/mL penicillin and 100 g/mL streptomycin). They were divided into
the Ad-duSOST treatment group and the Ad-NC control group, with three replicates in each group. Growing myoblasts (50% confluent) were infected with optimal MOI of Ad-duSOST and Ad-NC for proliferation assay. At 24 h post-infected, cells were harvested for sampling. The culture medium was changed to a differentiation medium (DM, DMEM/F12 containing 2% horse serum and 1% penicillin/streptomycin) until approximately 80% confluence was reached. The optimal MOI of Ad-duSOST and Ad-NC virus solution was added to each group. All cells were cultured in the incubator with 5% CO2 at 37 °C. The cell samples infected by the virus were collected and stored at −80 °C for RNA isolation.

Cell proliferation assay

Myoblasts viability was analyzed by a Cell Counting Kit-8 (CCK8, Bestbio Biotechnology, China). The primary myoblasts were seeded into a 96-well plate with a density of 5×103 cells/well; after transfection Ad-duSOST or Ad-NC, myoblasts were incubated with 10 μL CCK-8 for additional 3 h at 37 °C; the absorbance at 450 nm was measured using a Microplate Reader (Thermo, USA). The samples from each treatment had six replicates at each time point.

Duck myoblasts were inoculated into 96-well plates with 2000–3000 cells per well with three repeats in each group. After 24 h of treatment, the cells were incubated

Fig. 1 Construction and identification of recombinant adenovirus vector expressing duck SOST. a A schematic route to show recombinant adenovirus vector expressing duck SOST by AdEasy adenovirus system; b PCR products of SOST coding region. c Identification of pAdTrack-CMV-duSOST plasmid by double enzyme digestion reactions (XhoI and KpnI). d Identification of pAd-duSOST plasmid by PacI enzyme digestion. M1–M2 is a 2000 and 10,000 bp DNA Marker. M3 is λ-Hind III digest
with EdU medium for 3 h and then fixed with 4% paraformaldehyde. After that, the test was carried out according to the instructions of the EdU kit (Ribobio, China). Finally, it was observed and photographed under a fluorescence microscope.

**Flow cytometry assay**

Total $1 \times 10^5$ myoblasts were inoculated in a 6-well plate and transfected when the cell density was about 40–50%. After 24 h of transfection, the cells were digested with trypsin and collected. Then, the cells were washed with PBS, centrifuged at 300 rpm for 5 min, and then discarded the supernatant. The precipitation was fixed with ice-precooled 70% ethanol at 4 °C for 18 h. The samples were centrifuged at 300 rpm for 5 min, and the precipitate at the bottom was suspended with 1 mL PBS and filtered with a 300-mesh sieve. After centrifugation for 5 min at 300 rpm, PBS was discarded, and the precipitate was stained with 0.5 mL PI solution for 15 min (37 °C, avoid light). The number of cells in each stage was analyzed by flow cytometry at 488 nm laser wavelength.

**Immunofluorescence**

Myoblasts were switched to differentiation medium (DM) for 2 days infected with Ad-duSOST or Ad-NC, and then these cells were used for immunofluorescence (IF) staining. The cells were fixed with 4% paraformaldehyde (PFA) for 30 min and treated with 0.1% Triton X-100 for 5 min at room temperature, then blocked with 5% BSA for 1 h. After incubation overnight at 4 °C in a mouse primary polyclonal antibodies against MYHC (diluted 1:500; Biosynthesis Biotechnology, China), the cells were incubated against Cy3-conjugated secondary antibody (diluted 1:500; Beyotime Biotechnology, China). To visualize the nuclei, the slides were co-stained with 4,6-diamidino-2-phenylindole (DAPI; Sigma). Then, the cells were visualized with an immunofluorescence microscope after being washed in PBS thrice. The relative area of positive staining was evaluated with Image-Pro Plus 6.0 software (Media Cybernetics Corporation, USA).

**Western blot**

Total proteins were isolated by lysing cells in ice-cold RIPA lysis buffer. Protein samples from individual experiments were pooled for Western blotting analysis. The primary antibodies were used; anti-MYHC (DSHB, USA, 1:500 dilutions) and anti-GAPDH (Beyotime, China, 1:1000 dilutions). The membranes were incubated with antibodies at 4 °C overnight and washed in washing buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 0.1% Tween 20). Next, the membranes were treated with horseradish peroxidase-conjugated IgG antibody (1:2000 dilutions) for 2 h at room temperature. Each experiment was biologically replicated for triplicate times. Densitometry analysis of the bands relative to GAPDH was performed using Image J software (National Health Institute, Bethesda, MD, USA).

**Real-time PCR**

Total RNA was extracted from myoblasts by RNAiso plus (Takara, Japan) according to the manufacturer’s instructions and then measured by spectrophotometry. RNA was reverse-transcribed to synthesize the cDNA using the reverse transcript system (Takara, China). Real-time PCR was carried out with the SYBR Prime Script RT-PCR Kit (Takara, China) using the Bio-Rad CFX Manager (Bio-Rad Laboratories, USA). Expression of duck SOST, CDK6, PCNA, and CCND1/2 were detected, β-actin and GAPDH were used as inner controls. One sample collected from cells was repeated in triplicate. Primer sequences for real-time PCR are provided in Table S3. The relative expression levels of each gene were analyzed by the $2^{-\Delta\Delta C_t}$ method [16].

**RNA-seq**

Two days’ treatment after the myoblasts transfected with Ad-duSOST and Ad-NC, the cells were collected for sampling. Each treatment has three replicate samples. RNA was isolated, and its concentration and integrity were measured. High-quality RNA was sent to Novogene (Beijing, China) for cDNA libraries construction and sequencing. RNA-seq libraries were then generated. Briefly, after RNA fragmentation, double-stranded cDNA was synthesized by replacing dTTPs (deoxythymidine triphosphate) with dUTPs (deoxuryridine triphosphate) in reaction buffer used for second-strand cDNA synthesis. The resulting double-stranded cDNA was ligated to adaptors after being end-repaired and A-tailed. Single-strand cDNA was then obtained using the USER (Uracil-Specific Excision Reagent) Enzyme (NEB, Ipswich, U.K.). Finally, PCR amplification was performed to enrich the cDNA libraries. Sequencing was performed on an Illumina Hiseq 2500 instrument to generate 150-bp paired-end reads.

**Mapping and differentially expressed genes (DEGs) analysis**

Hisat2 (v2.1.0) built the index of genome files and mapped RNA-seq data to the reference genome (IASCAAS_PekingDuck_PBH1.5, GCF_003850225.1). StringTie (v1.3.3b) assembles the alignments into full and partial transcripts, estimates the expression levels of all genes and transcripts, and creates read in files for Ballgown. Finally,
the software packages in R (v3.5.1), such as gene filter, dplyr, and devtools, were used to analyze the differential genes. Functional groups and pathways encompassing the DEGs were identified based on GeneOntology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the KOBAS 3.0 software. The threshold was set as a P ≤ 0.05.

**Statistical analysis**

The data were subjected to analysis of variance (ANOVA), and the means were compared for significance by Tukey’s test using SPSS22 (IBM Corp, Armonk, NY). The results were expressed as the mean ± SEM. A P-value of less than 0.05 was considered statistically significant.

**Results**

**Verification about the construction process of Ad-duSOST**

The PCR amplification was carried out according to the primers listed in Table S1, and the PCR product of duck SOST with a length of 750 bp was obtained (Fig. 1b). Then, the PCR product was constructed into pAdTrack-CMV, and the positive clones were identified by double enzyme digestion with XhoI and KpnI. Two fragments were obtained, about 750 bp and 10 kb, respectively (Fig. 1c). The sequencing works verified that the recombinant plasmid pAdTrack-CMV-duSOST had been constructed successfully, and afterward, they were co-transformed with pAdEasy-1 into BJ5183 (Fig. 1a). Later the recombinant plasmid was digested by PacI, and smaller and larger fragments were obtained with a length of 3 kb and 23.13 kb, respectively (Fig. 1d). The sequence analysis verified that the recombinant adenovirus plasmid pAd-duSOST was successfully constructed. Then, the pAd-duSOST was packaged in HEK293 cells to gain infection activities. The titer of recombinant adenovirus Ad-duSOST was 1 × 10^8 U/mL, tested by the LaSRT method (Fig. S1).

Duck myoblasts were infected by Ad-duSOST with MOI of 50, 60, 70, 80, and 90, respectively. After infection for 48 h, the mRNA expression of SOST in myoblasts was measured by Real-time PCR (Fig. S2). The expression of SOST in myoblasts with different MOI was significantly higher than that of the Ad-NC group (P < 0.05). In addition, the mRNA expression of SOST in myoblasts with 80 MOI was significantly higher than that in myoblasts with 70 MOI (P < 0.05).

**Effect of SOST on the proliferation of duck myoblasts**

To investigate the effects of SOST on myoblasts’ proliferation, the AD-duSOST was transfected into duck primary myoblasts. The CCK-8 cell proliferation assay showed that the cell proliferation activities in the treatment group were inhibited after 12, 36, and 48 h treatment, respectively (Fig. 2a). The EdU can bind to single-strand DNA and specifically label the nuclei in the S phase of the cell cycle. The EdU assay showed that the nuclear labeled by Hoechst in myoblasts treated with Ad-duSOST was lower than that of the Ad-NC group. The labeling rates of EdU positive cells in the treatment group were significantly lower than the Ad-NC group (P < 0.05, Fig. 2b). In addition, flow cytometry showed that most myoblasts were blocked in the G0/G1 phase (Fig. 2c). There was no significant difference in the number of G0/G1 cells between the treatment group and the Ad-NC group. The regulated factors of CDK6, PCNA, CCND1, and CCND2 were closely related to cell proliferation. Real-time PCR showed that all of them did not change under the treatments of Ad-duSOST (Fig. 2d).

**Effect of SOST on the differentiation of duck myoblasts**

The mRNA expression level of SOST in the myoblasts transfected with Ad-duSOST was significantly higher than that of the Ad-NC group. The SOST expressed to reach the maximum in myoblasts on the 2nd day (D2) after transfection, whereas it gradually decreased on 3rd (D3) and 4th (D4; Fig. 3a). Subsequently, immunofluorescence results showed that the fluorescence activity of MYHC decreased, and myotube formation was blocked by overexpressing SOST (Fig. 3b). MYHC is a marker gene of satellite cell differentiation, and its expression will significantly increase when the satellite cell begins to differentiate [17]. MYHC was detected by Western blot (Fig. 3c), and results showed that its protein level was decreased in myoblasts of the Ad-duSOST group than the Ad-NC group (P > 0.05).

**RNA-seq showed the main functional changes in myoblasts with SOST overexpression**

To further study the molecular changes in myoblasts during the differentiation process induced by overexpression of the SOST, the transcriptome profiling of myoblasts was compared between the Ad-duSOST infection group and the Ad-NC group. The details of RNA sequencing data reflecting the quality of sequencing works are provided in Table S4. The correlation analysis based on the TPM value of differentially expressed genes (DEGs) showed that the internal consistency of replicate samples in the same
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The genes involved in myoblast differentiation under the impacts of overexpression SOST

To further understand the role of the SOST in myoblast, we found the top 20 DEGs (P < 0.05, |Log2FC| > 3). Among them, ten genes were up-and down-regulated genes, respectively (Table S5). Through checking the functions of these twenty genes according to Genecards database (https://www.genecards.org/), it was found that these genes were mainly related to morphogenesis (NPS, BHLHA9, CLDN18, TAC1, ADA2, and FRK), disease (MYRFL, KLHDC7A, and NPY2R), and reproduction (ODF3, CRHBP, SPAM1, and DRC7). Among these genes, NPS has the highest Log2FC except MYRFL, promoting myoblast differentiation [20]. Moreover, some genes related to myogenic differentiation (according to literature) were enriched (Table 1). Among them, BMP2 [21], SOX6, and TGFBR3 [22] were reported to inhibit the differentiation of myoblasts, while β-catenin (CTNNB1) was to promote the myoblasts’ differentiation [23]. Moreover, BMP2, SOX6, and TGFBR3 were up-regulated, and CTNNB1 was down-regulated. Afterward, the PPI analysis was performed based on these 14 genes and found direct interaction of the SOST with CTNNB1 and BMP2 (Fig. 5).

Discussion

Both bone and muscle tissues are originated from mesenchymal stem cells in mesoderm during embryogenesis [1]. Studies on meat ducks have found that the sternum affects
the development of corresponding pectoral muscles in the process of calcification. Moreover, bones and muscles are the largest endocrine organs in animals, synthesizing and secreting various cytokines to affect each other’s development processes through paracrine pathways [4]. The SOST, a protein secreted by mature osteocytes, is a negative regulator of bone formation [24]. In duck, the higher expression of the SOST was found in the sternum, whereas lower expression was found in the pectoral muscle. Some reports suggested that the SOST may affect muscle development [13, 25, 26]. In this study, we observed the morphology of myoblasts at the cellular level. We found that SOST inhibited the differentiation of myoblasts, implying that the SOST may have multi-effect functions regulating the co-development of duck sternum and skeletal muscle.

Muscle development is complex, including myoblast proliferation, myotube formation, and muscle fiber differentiation. In duck, we found that the SOST had an inhibition effect on myotube formation. Similarly, transcriptome revealed that the DEGs were mainly enriched in regulation of the multicellular organismal process, regulation of cell differentiation, and cell developmental process, as well as, six of the top 20 DEGs were related to morphogenesis. Studies showed that the SOST also has inhibition effects on osteoblast differentiation. When the SOST binds to the LRP5/6 and Frizzled co-receptors on the cell surface of osteoblasts, Wnt/β-catenin signaling was inhibited [27], thereby inhibiting osteoblast differentiation [24, 28]. The difference is that the SOST may inhibit osteoblast differentiation and promote apoptosis and inhibit cell proliferation in bone [10, 11]. In vitro, the proliferation activity of MG63 cells was increased after silencing the expression of SOST [29]. In this study, we also verified the effect of SOST on the proliferation of myoblasts. However, flow cytometry results

Fig. 3 Effects of overexpression SOST on myoblasts’ differentiation. a The mRNA expression profiles of SOST in myoblasts after transfected with Ad-duSOST. b The immunofluorescence was used to detect the MYHC in the myoblast. The scale bar is 50 μm. c The protein content of MYHC, detected by Western blot, and GAPDH was used as the reference protein.
showed that the SOST overexpression did not inhibit cell cycle transition.

The mechanism of the SOST effect on the myoblasts differentiation is mainly related to the following pathways, such as Cytokine–cytokine receptor interaction, regulation of actin cytoskeleton, cAMP signaling pathway, and Wnt signaling pathway, which were reported to be involved in cell differentiation [19, 30, 31]. We compiled a series of marker genes during myoblast differentiation, including myogenic regulatory factors (MRFs) family, myocyte enhancer factor 2 (MEF2) family, Pax3/7, and Myostatin (MSTN). The results showed that their P-value or Fold-changes were not within the range of DEGs. There are various reports indicate different DEGs and their role in cell differentiation. Up-regulated genes, such as BMP2, SOX6, and TGFBR3, were reported to inhibit the differentiation of myoblasts [18, 20, 22, 32]. On the contrary, the down-regulated genes, such as β-catenin (CTNNB1), were reported to promote the differentiation of myoblasts [23]. Moreover, protein interaction network analysis showed that the SOST could directly interact with BMP2 and CTNNB1. Related studies also showed that the SOST

Table 1 The DEGs related to myoblast differentiation under the impacts of SOST overexpression

| Genes      | LOG2FC   | P value  |
|------------|----------|----------|
| BMP2       | 1.17996  | 0.07269  |
| BMPR1B     | 0.76732  | 0.00262  |
| GDF10      | 0.92765  | 0.04835  |
| TGFBR3     | 1.12352  | 0.02167  |
| ACVR1      | 0.99487  | 0.01038  |
| FZD10      | 1.87914  | 0.00869  |
| CTNNB1     | −2.21867 | 0.03428  |
| GRIN2B     | −1.12969 | 0.00587  |
| ADORA1     | 1.26710  | 0.01187  |
| MYOC       | −1.07702 | 0.00208  |
| SOX6       | 2.51936  | 0.00208  |
| CBLN4      | −1.18442 | 0.47288  |
| GATA4      | −1.20435 | 0.25342  |
| INHBB      | 0.75833  | 0.04666  |

TGFBR3 and CTNNB1 differed in transcripts.
could interact with BMP2 and CTNNB1 during osteoblast differentiation [33]. Therefore, we suspected that BMP2 and CTNNB1 might be involved in the process of SOST inhibiting myoblast differentiation.

Adenovirus vector is one of the most widely used viral vectors in the study of gene function, as its efficient gene transfer and protein expression in a variety of cells [34]. The shuttle plasmid contains a sequence encoding GFP, convenient for tracking and detection [35]. At present, there have been related studies to explore its role in osteoblasts by constructing adenovirus vectors that overexpress or silence SOST [29]. However, the possible role of SOST in the co-development of the duck sternum and pectoral muscle has not been reported. In the present study, we have constructed, for the first time, the adenovirus vector of duck SOST and observed its effect on duck myoblasts.

The present study constructed the recombinant adenovirus vector expressing duck SOST successfully. We have confirmed that the formation of myotubes may be inhibited by SOST overexpression. Multiple genes and pathways were significantly involved in this process, including 14 genes such as CTNNB1 and BMP2 with a fundamental change of regulation modes. It is suggested that SOST may be a critical gene regulating the co-development of the duck sternum and pectoral muscle.

Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s11033-022-07151-4.

Author contributions  HL and YL: methodology, visualization, writing an original draft. HL: investigation, methodology. QX: visualization. JW: visualization. CH: writing-review & editing. LB: writing-review & editing. LL: writing-review & editing.

Funding  This work was supported by the National Natural Science Foundation of China (31872345), Key Technology Support Program of Sichuan Province (2016NYZ0044), Agriculture Research System of China (CARS-43-6), and New breeding projects.

Data availability  The data that supports the findings of this study are available in the supplementary material of this article.

Declarations

Conflict of interest  The authors declare that they have no conflicts of interest with the contents of this article.

Ethical approval  The animal use protocol listed below has been reviewed and approved by the Sichuan Agricultural University Animal Ethical and Welfare Committee.

Consent to participate  All authors are acquainted with the manuscript.

Consent for publication  Not applicable.

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