MOLECULAR AND CELLULAR

Roles of chicken growth hormone receptor antisense transcript in chicken muscle development and myoblast differentiation

H. D. Xu,*2 T. Li,*2 Z. Wang,* P. Adu-Asiamah,* Q. Y. Leng,* J. H. Zheng,* Z. H. Zhao,* L. L. An,* X. Q. Zhang,† and L. Zhang*1

*Agricultural College, Guangdong Ocean University, Zhanjiang 524088, PR China; and †Guangdong Provincial Key Laboratory of Agro-Animal Genomics and Molecular Breeding, Key Laboratory of Chicken Genetics, Breeding, and Reproduction, Ministry of Agriculture, South China Agricultural University, Guangzhou 510642, PR China

ABSTRACT  Muscle is one of the important economic traits in poultry production, and its production depends on the increased number of muscle fibers during the embryonic stage. Chicken GHR gene can transcribe in double directions, possessing not only GHR-S but also GHR-AS. The 2 kinds of transcripts are partially complementation in sequences and interact with each other. Until now, the roles and mechanisms of GHR-AS in myoblast differentiation was still unknown. In this study, we not only analyzed the GHR-AS expression patterns in myoblast differentiation phase but also clarified that GHR-AS promoted myoblast differentiation via GH-GHR-IGF1 signal pathway. Quantitative PCR analysis indicated that GHR-AS was increased during myoblast differentiation. Sub-cellular localization showed that GHR-AS and GHR-S were expressed at a higher level in the nucleus than that in the cytoplasm. The expression of MyoD and MyHC and the myoblast differentiation significantly increased after GHR-AS overexpression, while the distance between wounds decreased, suggesting that GHR-AS repressed myoblast migration and promoted differentiation. Additionally, the expression of GHR-AS, IGF1 and MyHC increased after GH protein treated, and the myoblast differentiation also increased. In conclusion, GHR-AS promoted myoblast differentiation by enhancing fusion and inhibiting migration possibly via GH-GHR-IGF1 signal pathway.

Key words: chicken, growth hormone receptor, natural antisense transcript, myoblast, differentiation

INTRODUCTION

The muscle growth is a complex process which depends on the increased number of muscle fibers (Buckingham et al., 2010; Guo et al., 2015). Once the growth phase is completed, the fiber numbers do not increase but the fiber volume increases (Buckingham et al., 2010). These processes influence meat quality of poultry and livestock. During myoblast differentiation from embryonic stem cell to myofiber in skeletal muscle development, genes play important roles in different stages (Luo, 2016). Recently, many researchers identified long non-coding RNAs such as linc-MD1 and hic-Dum, in the regulation of myogenesis (Legnini et al., 2014; Wang et al., 2015). Natural antisense transcripts (NATs), a kind of non-coding RNAs, often overlapped with the protein coding gene transcripts in opposite directions and regulated the mRNA and protein expression (Faghihi and Wahlestedt, 2009).

GHR plays a key role in human and animal growth. Both human Laron-type dwarfism (Godowski et al., 1989) and sex-linked dwarf chicken (Lin et al., 2012) are caused by the mutation of GHR gene. Chicken GHR gene located in chromosome Z comprises of 8 exons. Growth hormone (GH) secreted by pituitary gland can combine with GHR to activate the intracellular insulin-like growth factors (IGFs) pathway involved in cell proliferation and differentiation (Sperling, 2016). Previously, we identified a NAT of GHR gene (GHR-AS), which was overlapped with GHR mRNA (GHR-S) in the 3’UTR, exons 8, 7, and 6 of GHR gene (Zhang et al., 2016).

HuaiXiang (HX) chicken is a famous local breed with yellow feathers, skin, and feet mostly found in the Guangdong province of China. Also, it has a high tolerance to roughage feeding and general stress resistance. In this study, we mainly analyzed the roles of GHR-AS in HX chicken myoblasts differentiation process, and...
found that GHR-AS promoted myoblast differentiation via the GH-GHR-IGF1 pathway.

MATERIALS AND METHODS

Ethics Statement

Animals involved in this research were humanely sacrificed as necessary to ameliorate suffering. It was approved by the Animal Care Committee of Guangdong Ocean University (Zhanjiang, China).

Experimental Animals and Tissue Samples

The fertile eggs were obtained from the HX Chicken Breeding Farm (Yingfu Company, Xinyi, Guangdong province), incubated in an automatic incubator (Baihui, Shandong, China) at 37.8°C, with 60 ± 10% humidity, and the broilers were fed with the normal basal diet, which including 11.51% metabolic energy, 15.23% crude protein, 3.10% calcium, 0.45% available phosphorus, and a few non-essential amino acid. According to our previous research (Zhang et al., 2017), E11 was the best time to isolate and culture chicken myoblast in vitro. Thus, the leg muscles collected from E11 embryos were used to isolate the chicken myoblast.

Isolation, Culture, and Differentiation of Chicken Primary Myoblast

Myoblasts were isolated from chicken leg muscles at E11 according to the methods described by Zhang et al. (2017). Briefly, differential attachment was performed 3 times at every 40 min after the muscles were cut into pieces. During the third time, the adherent cells were collected and directly grown on a plastic plate. The cells were cultured in growth medium with DMEM-F12 (Gibco, New York, USA), 15% FBS (Gibco, New York, USA) and 1% penicillin/streptomycin (Gibco, New York, USA) at 37°C in 5% CO2, then induced to differentiation in differentiation medium (DM), DMEM-F12 containing 2% horse serum (Gibco, New York, USA), until 90% confluent were reached.

Subcellular Location of GHR-AS in Chicken Myoblast

Nucleus and cytoplasm RNA were individually extracted from chicken myoblast during proliferation or differentiation phase according to the guide of PARIS Kit Protein and RNA Isolation System (Invitrogen, Carlsbad, CA, USA). The PARIS method is based on differential lysis of plasma and nuclear cell membranes by nonionic detergents. Cells are first separated into nuclear and cytoplasmic fractions then RNA is isolated. Briefly, the cell pellet was suspended in a buffer of RNA purification and then centrifuged twice at 4°C for 5 min. The supernatant was the cytoplasmic fraction and the pellet was the nucleus fraction. RNAs were extracted from both fractions using Trizol (Mange, Guangzhou, China). The effectiveness of cell fractionation can be easily checked by Quantitative PCR (qRT-PCR) or western blot with antibodies for proteins found predominantly in the nucleus or cytoplasm of cells such as sno-U6 (predominantly in the nucleus) or GAPDH (predominantly in the cytoplasm). In this study, we analyzed the sno-U6 and GAPDH expression with qRT-PCR to indicate the methodology was successful. The relative levels of GHR-AS and GHR-S were analyzed by qRT-PCR.

Construction of Chicken GHR-AS Plasmids and Cell Transfection

To construct the GHR-AS plasmid (pGHR-AS), the full length of GHR-AS was amplified by PCR with forward and reward primers including KpnI and XbaI restriction enzyme site at 5′-ends, respectively (Table 1). The PCR was performed with a 20 μL reaction mixture containing 1 μL of cDNA from chicken myoblast, 10 μL of TaKaRa Taq Version 2.0 plus dye (TaKaRa, Kyoto, Japan), 1 μL each of the forward and reward primer (10 μM), and double-distilled water. The PCR reaction procedure involved: 94°C 2 min, 40 cycles at 94°C 30 s, 57°C 30 s, and 72°C 30 s; followed by 72°C for 5 min. The PCR products were digested with KpnI and XbaI restriction enzyme and the purified fragment was ligated into pcDNA3.1(+)-plasmid to obtain the GHR-AS recombinant plasmid. GHR-AS overexpression experiment were carried out in a 12-well plate by transient transfecting pGHR-AS into myoblast during differentiation using Lipofectamine3000 (Invitrogen, Carlsbad, CA).

Wound-Healing Assay to Detect the Effects of GHR-AS on Myoblast Migration

A wound healing assay was carried out to compare the myoblast migration after being transfected with pGHR-AS or pcDNA3.1(+) plasmids. The transfected cells were cultured in a 12-well plate until confluent. A straight scratch was created in the center of each well using a micropipette tip. Cell migration was assessed by measuring the movement of the cells into the scratch in the well. After being incubated for 6 h, cells were photographed under an inverted microscope (Olympus, Tokyo, Japan). The distance between the 2 edges of the scratch (wound width) was measured at 9 sites (100 × magnification) in each image. The cell migration distance was measured using ImageJ software by measuring the wound width, and then normalized to the length at 0 h. Experiments were carried out 3 times.

Myoblast Treatment with Chicken GH Protein

After inducing myoblast differentiation, we added recombinant chicken GH (Abcam, Cambridge, London,
Table 1. Primers used in this study.

| Application                  | Primer name | Primer sequence                  | Product(bp) | Tm/°C |
|------------------------------|-------------|----------------------------------|-------------|-------|
| Constructed GHR-AS vector    | GHR-AS-F    | CTTggtaccGTATGAAGAGTCCCAACCAAC   | 4,184       | 57.0  |
|                              | GHR-AS-R    | CCCctctagaTACACGGGACTACAAGGAGGAC |             |       |
| GHR-AS quantitative PCR      | GHR-AS-F    | TTGCTAATGTGTTCTGGTCTGGT          | 117         | 59.9  |
|                              | GHR-AS-R    | GGGTCAATCCCTTTAATCTTT            |             |       |
| GHR-S quantitative PCR       | GHR-S-F     | AGTCCGATCAAGACAGCGTAC            | 128         | 59.9  |
|                              | GHR-S-R     | CTAAAGACAGGGAACACCAGG            |             |       |
| IGF1 quantitative PCR        | IGF1-F      | TGTGCTCCAATAAAGGACACCT          | 125         | 57.0  |
|                              | IGF1-R      | TTCTGCTTTTCTGTGTGTTCTCTAC        |             |       |
| MyoD quantitative PCR        | MyoD-F      | GCTACTACACGGAATCACCAAAT          | 200         | 59.8  |
|                              | MyoD-R      | CTGGGCTTCCACTGTCACTCA            |             |       |
| MyHC quantitative PCR        | MyHC-F      | CTACCTACAGGGAATCAGAAT            | 208         | 59.8  |
|                              | MyHC-R      | TGATAGTCGTATGGGTTGGT             |             |       |
| Myomaker quantitative PCR     | Myomaker-F  | GCTCTACAGGGAATCAGAAT            | 135         | 58.0  |
|                              | Myomaker-R  | CTGGGCTTCCACTGTCACTCA            |             |       |
| Internal control in qPCR analysis | GAPDH-F  | AGGACCCAGGTGCTCTCCTGT          | 153         | 60.0  |
|                              | GAPDH-R     | CCAATCAAGTCCACAAACCGG           |             |       |
|                              | Sno-U6-F    | CTCGCTTTCGCCAGCACAA             | 94          | 53.0  |
|                              | Sno-U6-R    | AAGGCTTCCAGAATTTGCGT            |             |       |

The lowercase part is the enzyme site.

UK) at 200 ng/mL in DM. Then we harvested cells for the analysis of IGF1 expression at 0, 3, 6, 9, 12, and 24 h, respectively.

**Quantitative Real Time PCR (qRT-PCR)**

The total RNA was extracted from myoblast by Trizol reagent (Mange, Guangzhou, China) according to the manufacturer’s protocol. The RNA integration and concentration were detected using electrophoresis at 1.5% agarose gel and Nanodrop 2000 spectrophotometry.

The cDNA was synthesized with PrimeScript RT Reagent Kit (TaKaRa, Kyoto, Japan). In addition, the cDNA was diluted 4 times with RNase free water and stored at −20°C. Real time PCR was conducted in a Bio-rad CFX96 Real-Time Detection System (Bio-rad, Hercules, CA, USA) using TransStart Green qPCR SuperMix (Transgen Co., Ltd., Beijing, China). The GHR-AS, GHR-S and IGF1 qRT-PCR primers used are listed in Table 1. The expressions were normalized with GAPDH, whiles gga-sno-U6 was used as the internal control of nucleus RNA. We performed qRT-PCR with 20 μL mixture containing 1 μL of cDNA, 10 μL of 2 × TransStart Green qPCR SuperMix, 0.5 μL each of the forward and reward primers (10 μM), and double-distilled water. The qRT-PCR reaction procedure is as follows: 95°C for 30 s; 40 cycles at 95°C for 5 s, annealing for 30 s, 72°C for 30 s; followed by 72°C for 5 min. The expression of gene was quantified using comparative threshold cycle (2^{−ΔΔC_{T}}) methods.

**Western Blot Analyzing the IGF1 Protein**

For Western blotting, cells were collected from different groups, then pelleted by centrifugation and lysed in RIPA lysis buffer (P0013B, Beyotime, Shanghai, China, https://www.beyotime.com/ripa-lysis-bufferh.htm) containing 1% Phenylmethanesulfonyl fluoride (ST506, Beyotime, Shanghai, China). Total protein was prepared and its concentration was determined using the BCA method (P0010, Beyotime, Shanghai, China). Protein was then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto the polyvinylidene fluoride (PVDF) membrane. After blocking for 1.5 h at room temperature with 5% skimmed milk, the PVDF membrane was incubated with anti-IGF1 (Cloud-Clone Corp, Wuhan, China), and anti-GAPDH antibodies (Bioworld, Nanjing, China), respectively. After washing 3 times with Tris-Buffered Saline and Tween-20, the membrane was incubated with horseradish peroxidase-conjugated secondary goat anti-rabbit IgG (H&L) antibody (Bioworld, Nanjing, China) at room temperature for 1.5 h, followed by visualization using SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific, Karlsruhe, Germany). An ImageMaster DVS system was used to calculate the relative mean gray values (A) of the target product and GAPDH. Expression index (I) of the target product was calculated using the formula of I = A product/A GAPDH.

**Immunofluorescence Assay**

Chicken primary myoblast at 95% confluence were washed 3 times with PBS (pH 7.4), and permeabilized for 15 min in PBS with 0.5% Triton X-100 before fixation in 4% paraformaldehyde for 30 min. Immunostaining was carried out as follows: the cells were incubated at 4°C overnight with primary anti-MyHC, diluted in 1% BSA. The cells were washed and then incubated at room temperature for 2 h.
Figure 1. The relative expression of GHR-AS and GHR-S gene expression during chicken myoblast differentiation. Each value represented the mean of 3 batches ± SEM.*P ≤ 0.05, **P ≤ 0.01.

Figure 2. Subcellular localization of GHR-AS and GHR-S in myoblast proliferation (A) and differentiation (B).

with the corresponding secondary antibody goat anti-rabbit IgG H&L (1:1,000; Abcam, Cambridge, MA) diluted in 1% BSA. DNA was visualized using 5 mg/mL DAPI. Finally, the cells were washed 3 times with PBS and observed with fluorescence microscope (DM5000B, Leica, Solms, Germany).

Statistical Analysis

Each experiment was repeated 3 times, and all the data were expressed as the mean±SE and processed using the statistical software SAS 9.1.3 (SAS Institute Inc., NC). *P < 0.05 was considered significant differences between groups.

RESULTS

GHR-AS Increasingly Expressed During Myoblast Differentiation

To investigate the expression of GHR-AS in chicken myoblast, primary myoblast was isolated from HX chicken leg muscles at E11, and induced differentiation by adding 2% horse serum to the DM. Quantitative PCR analysis indicated that GHR-AS was increased during myoblast differentiation. Also, the GHR-S has the same trend with GHR-AS, suggesting they might function during myoblast differentiation (Figure 1).

GHR-AS was Expressed at a Higher Level in the Nucleus than in Cytoplasm

To understand the possible roles and mechanisms of GHR-AS during myoblast proliferation and differentiation, we analyzed the location of GHR-AS using the PARIS RNA Isolation System Kit. Results showed that GHR-AS was rich in nucleus than cytoplasm in both myoblast proliferation (Figure 2A) and differentiation (Figure 2B). GHR-S possesses the same trend with GHR-AS. The nucleus co-localization of both GHR-AS and GHR-S demonstrated that they could interact with each other.

GHR-AS Repressed Myoblast Migration and Promoted its Differentiation

To evaluate roles of GHR-AS in myoblast differentiation and fusion, the myoblast was transfected with pGHR-AS during differentiation. Compared to the
control group, the myoblast differentiation and the expression of MyHC, MyoD, and Myomaker significantly increased (Figure 3A). Immunofluorescence assay for MyHC indicated that the differentiation of chicken myoblast were promoted (Figure 3B). Also, GHR-S was increased during the process (Figure 3C).

Migration is the important character to measure the ability of cell differentiation. Scratch wound healing assay showed that the distance between wounds in GHR-AS overexpression group was larger than the control group (Figures 4A and 4B), indicating that GHR-AS inhibited myoblast migration.
GHR-AS May Regulate the Myoblast Differentiation via GH-GHR-IGF1 Signal Pathway

Animal growth and development are affected by GH-GHR-IGF1 signal pathway: GH protein secreted from the pituitary gland briefly binds to the GHR in cytomembrane and activates the GH-GHR-IGF1 signaling pathway. Other tissues, like genital gland, also secrete GH protein, promoting IGFs expression by paracrine or autocrine fashion (Luna et al., 2014; Marisela et al., 2016).
Figure 5. The effects of chicken GH protein on myoblast differentiation. (A): the relative expression of \( IGF1 \) mRNA and protein in myoblast differentiation phase after GH treated; (B): the relative expression of \( MyHC \) mRNA, the variation of myoblast fusion index, and immunofluorescence of \( MyHC \) in myoblast differentiation phase after GH treated; (C): the relative expression of \( GHR-AS \) and \( GHR-S \) in myoblast differentiation phase after GH treated. Each value represented the mean of 3 batches±SEM. *\( P \leq 0.05 \), **\( P \leq 0.01 \).

To evaluate the function of \( GHR-AS \) via GH-GHR-IGF1 signal pathway, chicken GH protein was added during myoblast differentiation. Firstly, the cells underwent serum starvation for 10 h, then, 200 ng/mL chicken GH protein was added into the DM to induce \( IGF1 \) expression. We found that \( IGF1 \) mRNA significantly increased at the 6 h after adding GH protein (Figure 5A). Also, the IGF1 protein showed an ascending trend in the GH treated group (Figure 5A). Accompanied with this, the myoblast fused into multinucleate cells and the differentiation biomarker \( MyHC \) was highly expressed than the control group (Figure 5B). Interestingly, we found that both \( GHR-AS \) and \( GHR-S \) showed upward trends during the process, suggesting that \( GHR-AS \) may be involved in the myoblast differentiation via GH-GHR-IGF1 signal pathway (Figure 5C).

DISCUSSION
Amount of chicken lncRNAs involved in muscle development have been found by RNA sequencing analysis (Lin et al., 2014; Li et al., 2016). In the
previous study, we identified a non-coding transcript of GHR gene, named GHR-AS, overlapping with GHR-S in a tail-to-tail manner. Previous research showed that GHR-AS had slight effects on myoblast proliferation (Zhang, 2016). During the period of E12 to E17, the vital time for muscle formation, the number of myocytes grow fast and lay the foundation for growing and thickening after birth (Li et al., 2017). Here, we explored the effects of GHR-AS on myoblast differentiation. In the myoblast differentiation progress, GHR-AS appeared in an ascendant tendency. This suggests that GHR-AS may be involved in myoblast differentiation. 

RNA distribution analysis showed that both GHR-AS and GHR-S were rich in nucleus than cytoplasm in myoblast proliferation as well as differentiation. Other research showed that GHR-AS could change the chromatin status from a closed and inactive state to an open and active state, regulating methylation of histone H3K9me2 on its promoter in LMH cell line (Zhang et al., 2016). The nucleus co-localization of both GHR-AS and GHR-S in myoblast demonstrated that GHR-AS may regulate GHR-S in the same way in myoblast.

The fusion of myoblast with the growing multinucleated muscle cell is essential in skeletal muscle growth and development (Sotiropoulos et al., 2006). When GHR-AS was overexpressed in chicken myoblast, MyHC, MyoD, and Myomaker increased significantly. Previous report indicated that the myoblast fusion was inhibited after the GHR gene being knocked down in mouse, and the cross-sectional area and the number of nucleus in myofiber were noted to be lower than the control group (Mavalli et al., 2010). In the GHR knocked down mouse, the power of gripping and staying in limbs was decreased (Mavalli et al., 2010). All these research indicated that GHR played vital roles in animal myoblast differentiation. It was noteworthy that GHR-S increased when GHR-AS was overexpressed. This reminds us that GHR-AS might function in myoblast differentiation via GHR-S and GH-GHR-IGF1 signal pathway.

Migration and fusion are essential parts in myoblast differentiation. Cell movement capacity in migration is higher than any stage of differentiation, and fusion is the progress of recognition and conglutination among cells (Tomé et al., 2011). Scratch wound healing assay revealed a significant delay in the migration of myoblast after overexpressing of GHR-AS, indicating its inhibitory action on cell migration. Collectively, these data suggested that GHR-AS could promote myoblast differentiation by accelerating fusion and inhibiting migration.

Animal growth and development are regulated by the endocrine growth axis. GH, secreted by pituitary gland, affects tissues and organ development via the GH-GHR-IGF1 signal pathway (Wu et al., 2007). In addition, tissues themselves, like genital gland, also secrete GH protein, promoting IGFs expression to regulate cell proliferation, tissue generation, and organism development by paracrine or autocrine manner (Luna et al., 2014; Marisela et al., 2016). Previous studies exhibited that GH protein can promote IGF1 expression in mouse C2C12 cell (Vincent et al., 2011) and chicken primary hepatocytes (Wang et al., 2013) in vitro. In this study, we added 200 ng/mL GH protein in differentiation medium to stimulate IGF1 expression. After treatment, we found that IGF1, MyHC, and the fusion index of myoblast were significantly higher than the control, indicating that the IGF1 promotes myoblast differentiation. Interestingly, we found that GHR-AS and GHR-S also increased after adding GH protein in DM. The results demonstrated that GHR-AS can promote myoblast differentiation via GH-GHR-IGF1 signal pathway.

The secretion of hormone, like GH, is impulse type in vivo, and treating myoblast cultured in vitro with GH in impulse type can promote IGF1 expression more than in continuous mode (Frost et al., 2002), in which there are negative feedback in their regulation pattern. In this study, the IGF1 expressed increased in 6 h but decreased in 12 h, which may be related to the negative feedback regulation.

All together, we concluded that chicken GHR-AS promotes myoblast differentiation possibly via GH-GHR-IGF1 signal pathway and interacting with GHR-S.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation (31672412), the Natural Science Foundation of Guangdong Provincial (2017A030307002), the Open Fund of Key Laboratory of Chicken Genetics, Breeding and Reproduction, Ministry of Agriculture (Guangzhou) (201501), and The Key Platform Projects of Innovative Strong School Engineering by Department of Education of Guangdong Province (2018302).

REFERENCES

Buckingham, M., L. Bajard, T. Chang, P. Daubas, J. Hadchouel, S. Meilhac, D. Montarras, D. Rocancourt, and F. Relaix. 2010. The formation of skeletal muscle: from somite to limb. J. Anat. 202:59–68.

Faghihi, M. A., and C. Wahlestedt. 2009. Regulatory roles of natural antisense transcripts. Nat. Rev. Mol. Cell Biol. 10:637–643.

Frost, R. A., G. J. Nystrom, and C. H. Lang. 2002. Regulation of IGF-I mRNA and signal transducers and activators of transcription-3 and -5 (Stat-3 and -5) by GH in C2C12 myoblasts. Endocrinology 143:492–503.

Godowski, P. J., D. W. Leung, L. R. Meacham, J. P. Galgani, R. Hellmoss, R. Keret, P. S. Rotwein, J. S. Parks, Z. Laron, and W. I. Wood. 1989. Characterization of the human growth hormone receptor gene and demonstration of a partial gene deletion in two patients with Laron-type dwarfism. PNAS 86:8083–8087.

Guo, B., P. L. Greenwood, L. M. Cafe, G. Zhou, W. Zhang, and B. P. Dalrymple. 2015. Transcriptome analysis of cattle muscle identifies potential markers for skeletal muscle growth rate and major cell types. BMC Genomics. 16:177. 16.1(2015-03-13).
Legnini, I., M. Morlando, A. Mangiavacchi, A. Fatica, and I. Bozzoni. 2014. A feedforward regulatory loop between HuR and the long noncoding RNA linc-MD1 controls early phases of myogenesis. Mol. Cell 53:506–514.

Li, H., J. Yang, X. Wei, C. Song, D. Dong, Y. Huang, X. Lan, M. Plath, C. Lei, and Y. Ma. 2017. CircFUT10 reduces proliferation and facilitates differentiation of myoblasts by sponging miR-133a. J. Cell. Physiol. 232:4643–4651.

Li, T., X. Mo, L. Fu, B. Xiao, and J. Guo. 2016. Molecular mechanisms of long noncoding RNAs on gastric cancer. Oncotarget 7:8601–8612.

Lin, S., H. Li, H. Mu, W. Luo, Y. Li, X. Jia, S. Wang, X. Jia, Q. Nie, Y. Li, and X. Zhang. 2012. Let-7b regulates the expression of the growth hormone receptor gene in deletion-type dwarf chickens. BMC Genomics 13:306.

Lin, X. C., Y. Zhu, W. B. Chen, L. W. Lin, D. H. Chen, J. R. Huang, K. Pan, Y. Lin, B. T. Wu, and Y. Dai. 2014. Integrated analysis of long non-coding RNAs and mRNA expression profiles reveals the potential role of lncRNAs in gastric cancer pathogenesis. Int. J. Oncol. 45:619–628.

Luna, M., C. G. Martínezmoreno, M. S. Ahumadasolórzano, S. Harvey, M. Carranza, and C. Arámburo. 2014. Extrapituitary growth hormone in the chicken reproductive system. Gen. Comp. Endocrinol. 203:60–68.

Luo, W. 2016. MicroRNAs-Mediated Signaling Pathways Regulate Chicken Myoblast Proliferation and Differentiation. South China Agricultural University, Guangzhou. Doctor.

Marisela, A.-S. S., C. G. Martínez-Moreno, M. Carranza, J. Ávila-Mendoza, J. L. Luna-Acosta, S. Harvey, M. Luna, and C. Arámburo. 2016. Autocrine/paracrine proliferative effect of ovarian GH and IGF-1 in chicken granulosa cell cultures. Gen. Comp. Endocrinol. 234:47–56.

Mavalli, M. D., D. J. Digiorlamo, Y. Fan, R. C. Riddle, K. S. Campbell, T. V. Groen, S. J. Frank, M. A. Sperling, K. A. Esser, and M. M. Bamman. 2010. Distinct growth hormone receptor signaling modes regulate skeletal muscle development and insulin sensitivity in mice. J. Clin. Invest. 120:4007–4020.

Sotiropoulos, A., M. Ohanna, C. Keckzia, R. K. Menon, J. J. Kopchick, P. A. Kelly, and M. Pende. 2006. Growth hormone promotes skeletal muscle cell fusion independent of insulin-like growth factor 1 up-regulation. PNAS 103:7315–7320.

Sperling, M. A. 2016. Traditional and novel aspects of the metabolic actions of growth hormone. Growth Horm. IGF Res. 28:69–75.

Tomé, M., P. Lópezromero, C. Albo, J. C. Sepúlveda, B. Fernándezgutiérrez, A. Dopazo, A. Bernad, and M. A. González. 2011. miR-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells. Cell Death Differ. 18:985–995.

Vincent, M., S. Selvakumari, D. T. Chiel, V. D. Paul, L. Patrick, M. C. Lamers, H. P. Harding, R. David, W. H. Lamers, and K. H. S Eleonore. 2011. Arginine deficiency causes runting in the suckling period by selectively activating the stress kinase GCN2. J. Biol. Chem. 286:8866–8874.

Wang, H., L. Yang, F. Shao, X. Wang, Y. U. Jianfeng, and G. U. Zhiliang. 2013. Research on GH regulation of GH/IGF-pathway related gene expression in primary chicken hepatocytes. J. Yangzhou Univ. Nat. Sci. Ed. 34:23–28.

Wang, L., Y. Zhao, X. Bao, X. Zhu, Y. K. Kwok, K. Sun, X. Chen, Y. Huang, R. Jauch, and M. A. Esteban. 2015. LncRNA Dum interacts with Dumts to regulate Dppa2 expression during myogenic differentiation and muscle regeneration. Cell Res. 25:335–350.

Wu, G. Q., J. X. Zheng, and N. Yang. 2007. Expression profiling of GH, GHR, and IGF-1 genes in sex-linked dwarf chickens. Hereditas 29:989–994.

Zhang, L. 2016. Research on the Function of Chicken GHR Natural Antisense Transcripts. South China Agricultural University, Guangzhou. Doctor.

Zhang, L., S. Lin, L. An, J. Ma, F. Qiu, R. Jia, Q. Nie, D. Zhang, Q. Luo, and T. Li. 2016. Chicken GHR natural antisense transcript regulates GHR mRNA in LMH cells. Oncotarget 7:73607–73617.

Zhang, L., Z. Wang, L. Ting, X. H. Dong, M. Xiao, M. Q. Liu, Q. H. Nie, X. Q. Zhang, and A. L. Long. 2017. The effect of GH protein on the expression of IGF1 in chicken (Gallus gallus) myoblast cells. J. Agric. Biotechnol. 25:511–516.