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■ Keywords

Tibetan chickens; Ovary; Transcriptome sequencing; lncRNA; mRNA.



Section editor: Irenilza A. Nääs

Submitted: 03/May/2024
Approved: 19/July/2024

Comparative Analysis of Long Noncoding RNA and mRNA Expression Profiles in Ovarian Tissues of Tibetan Chickens and Roman Chickens During the Egg Laying Period

ABSTRACT

To explore the important long noncoding RNA (lncRNA) and its target genes in Tibetan chicken ovarian tissue, whole transcriptome sequencing technology and bioinformatics methods were used to analyze and predict lncRNA, differential expression of mRNA, and lncRNA between the two different breeds using DESeq2 software. The study predicted the Antisense and Cis regulatory target genes of differentially expressed lncRNA and performed functional annotation of GO, and analysis of the KEGG signaling pathway in these target genes. Quantitative fluorescence PCR was conducted in real time to validate the expression of important target genes. The results showed the discovery of 532 differentially expressed lncRNAs, 340 of which upregulated and 292 downregulated, as well as 2314 differentially expressed mRNAs, with 983 being upregulated and 1331 downregulated. The differentially expressed lncRNAs were found to regulate 48 differentially expressed mRNAs in the sense direction, and 14 in the antisense direction. Enrichment analysis on the intersection of target genes of differentially expressed lncRNAs and mRNAs showed enrichment mainly in KEGG signaling pathways such as TGF- β signaling pathway (ko04350), organic selenium compound metabolism (ko00450), and oocyte meiosis (ko04114). The quantitative fluorescence PCR validation of important target genes such as CYP17A1, PTPN5, ACSL3, Nf2, CYP11A1 showed consistent results with RNA-seq results. This indicates the presence of lncRNA and mRNA with different expression levels and specific expression in the ovarian tissues of Tibetan and Roman chickens, and that genes such as CYP17A1, PTPN5, ACSL3, Nf2, and CYP11A1 may be key factors regulating the laying performance of Tibetan chickens.

INTRODUCTION

Long noncoding RNAs (lncRNAs) are a class of RNA molecules that lack protein coding ability and have a length ranging from 200 to 100,000 nt, located in the cytoplasm or cell nucleus (Cao *et al.*, 2006; Mercer *et al.*, 2009). Compared to messenger RNA (mRNA), lncRNAs exhibit stronger tissue-specific expression patterns, and play an indispensable roles in cell-type-specific processes (Zuckerman & Ulitsky. 2019). With the advancement of high-throughput sequencing technologies and deepening research on noncoding RNAs, it has been discovered that lncRNAs can actually translate certain peptides with lengths typically less than 100 amino acids. These short peptides also play diverse roles in organisms, such as involvement in X chromosome silencing, genomic imprinting, chromatin modification, transcriptional activation, transcriptional interference, nuclear transport, and other crucial regulatory processes (Ohhata *et al.*, 2008). lncRNAs are no longer considered as “noise” in genome transcription, but rather possess significant biological functions (Taylor *et al.*, 2015; Liu *et al.*,



2022). For example, in the regulation of reproductive function, studies have shown that lncRNAs play important regulatory roles in various reproductive processes, such as human sperm formation and maturation, sperm vitality and morphology, follicle development and maturation, and embryonic development and implantation (Zhang *et al.*, 2024). They also impact estrogen synthesis in granulosa cells of mice (Hu *et al.*, 2023), participate extensively in goat follicle development (Xu *et al.*, 2021), granulosa cell proliferation, oocyte formation, and regulate FSH expression levels in ducks (Ren *et al.*, 2017).

Egg-laying performance is a crucial economic trait in the poultry industry, where egg production is the most direct indicator of egg-laying performance (Huang *et al.*, 2022). The growth and development levels of the ovary and follicles directly influence the egg production of poultry. The ovary is a unique and essential reproductive organ and endocrine gland, and its development is a complex and highly coordinated biological process. It is regulated by a large number of genes at the transcriptional level, while relying on the combined participation of various hormones, functional proteins, and regulatory factors. The physiological functions of the ovary are exerted through relevant signaling pathways and regulatory mechanisms (Saleh *et al.*, 2021). Differential types of gene or lncRNA expression in ovarian tissue and their changes in expression levels at the molecular level affect the reproductive performance of poultry. Roman chickens, being one of the most extensively bred commercial layer breeds globally, exhibit exceptional production performance, characterized by an early onset of laying and a high laying rate (Zhang *et al.*, 2012). The Tibetan chicken breed is primarily found in Tibet, and Qinghai, and Sichuan provinces. They exhibit small body size, limited production performance, and low laying rates (Li *et al.*, 2023). Having undergone long-term natural selection, Tibetan chickens exhibit clear advantages over commercial breeds in terms of adaptability, disease resistance, and product quality. However, due to the lack of intensive artificial selection, egg-laying performance is significantly lower than that of commercial breeds. This study uses analysis of transcriptome sequencing data from ovarian tissue during the peak laying period of Tibetan chickens and Roman egg-laying chickens to obtain the expression profiles of coding and non-coding RNAs in ovarian tissue. It also constructs a regulatory network of lncRNA-mRNA related to ovary development, screens candidate genes that regulate the egg-laying period

of Tibetan chickens, and analyzes their regulatory network to provide breeding references for further breeding of Tibetan chicken breeds with high egg-laying performance.

MATERIALS AND METHODS

Experimental Animals

The experiment was carried out in the internship pasture of the Tibet Institute of Agriculture and Animal Husbandry in Bayi District, Linzhi City, Tibet Autonomous Region. Tibetan and Roman chickens, both at approximately 300 days of age, were procured from Tibet Linzhi Hongyu Breeding Development Co., Ltd. and Tibet Lhasa Qushui Sanyou Jingtian Product Development Co., Ltd., respectively. Following a one-month period of co-housing in cages under identical feeding conditions, nine healthy chickens from each breed were randomly selected to form three biological replicates with three each. Tibetan chickens were designated as the TC group (Tibetan Chicken) and their samples were labeled TC1, TC2, and TC3; and Roman chickens were designated as the RC group (Roman Chicken), with samples labeled RC1, RC2, and RC3. After the chickens were slaughtered, their ovarian tissues were collected and stored in liquid nitrogen for later use.

Total RNA Extraction and Library Construction

After total RNA was extracted from the samples, ribosomal RNA was removed to maximize the retention of all coding and noncoding RNA. Quality evaluation of RNA, library construction, and sequencing was carried out by Guangzhou Kidbio Biotechnology Co., Ltd. using the Illumina HiSeq™ 4000 system.

Sequencing Data Quality Control, Analysis, and Prediction

The original sequencing data (raw reads) were subjected to quality control using Fastp (Chen *et al.*, 2018) to obtain clean reads. After strict quality control, optimized data was obtained and alignment analysis based on the reference genome of the *Gallus gallus* species (Ensembl_release106) was performed using the HISAT2 software. The transcripts were reconstructed using String Tie, and three software programs (CPC2, CNCI, Feeln) were utilized to predict the coding potential of new transcripts. The intersection of transcripts without coding potential was considered reliable prediction of lncRNA. Antisense binding



between LncRNA and mRNA was predicted using RNAplex, and cis-acting LncRNA within 10kb upstream and downstream of a gene was predicted using Bedtools software. The DESeq2 software was used for differential analysis of genes and transcripts, with the filtering conditions $FDR < 0.05$ and $|\log_2FC| > 1$ to obtain differential LncRNAs and mRNAs. Functional enrichment analysis of LncRNA target genes was performed using GO and KEGG analysis to predict the main functions of LncRNA. The analysis of the association of LncRNA and mRNA was performed using the Omicsmart platform (<https://www.omicsmart.com/>) to generate an interaction network diagram.

Real-time Fluorescent Quantitative PCR Identification

To validate the sequencing results, one pair of LncRNA-mRNA with Antisense, Cis, and the highest Pearson's correlation coefficient in Trans action was selected for validation analysis using RT-qPCR. The primers were designed using the Primer 5.0 software,

based on chicken gene sequences published in Gene Bank, and LncRNA sequences obtained from sequencing splicing, with β -actin as the reference gene. The primers were synthesized by Wuhan Jinkairui Biotechnology Co., Ltd. (Table 1). RT-qPCR was performed following the operating instructions of the fluorescence quantitative PCR reagent kit from Wuhan Saiweier Biotechnology Co., Ltd., with a reaction system of 20 μ L. Quantitative RT-PCR data of mRNA levels were calculated using the comparative CT method (also known as the $2^{-\Delta\Delta CT}$ method) (Schmittgen *et al.*, 2008).

Statistical analysis

The experimental results were expressed as "mean $\pm$ standard deviation." Significance comparisons between different groups were performed using T tests in IBM SPSS Statistics 26.0. A p -value > 0.05 indicated that there were no significant differences, $p < 0.05$ indicated a significant difference, and $p < 0.01$ indicated an extremely significant difference.

Table 1 – Primers information.

Genes	Primer sequences(5'→3')	Product length, bp	Accession No.
<i>PTPN5</i>	F:AATGGATGAAGCTGAAAGGCAA R:CTGAGATGGTCCCATAGTGCTGTA	200	XM_046942031.1
<i>CYP17A1</i>	F:CCCTACCTGGAGGCTACCAT R:CGGACCAGAGGTTGATGACC	145	NM_001001901.3
<i>ACSL3</i>	F:GAAATCCAGCCATCAGGAAAGG R:GACCCAACACTGCTAACCCATT	121	XM_015277033.4
<i>Nf2</i>	F:AGAGGAACCTGTACCTTTCACTT R:TCACCATACTTGGCTTGGACTG	192	NM_204497.2
<i>CYP11A1</i>	F:AGGGAGAAGTTGGGTGTCTACGA R:CTTGTTCGGTAGTCACGGTAT	138	NM_001001756.2
ENSGALT00000099103	F:ACCTGGTCCACGGGTCACCTAG R:CAGCAGCAGAAAGTGGTGGTTAC	128	
ENSGALT00000098365	F:CACTGCCACTTCCTCTGATTTC R:GCTCAGATGACTGCTAATGGGTT	144	
MSTRG.18681.2	F:CTCACCTCAGCATAACATGAACAAC R:ACTAGTGCTTGCCTTGGTTGAA	200	
MSTRG.21492.1	F:TTTCATTGTGCCACTACTTGC R:GAAGGACGGAGCAGCGATT	228	
MSTRG.10917.2	F:CACAAATGAAGCAAAGGGCAG R:TCCGACCCACCTGGAATAA	160	
β -actin	F:CTGACTGACCGCTTACTCC R:TTGCACATACCGAGCCATT	84	NM_205518.1

PTPN5: protein tyrosine phosphatase, nonreceptor type 5; *CYP17A1*: cytochrome P450 family 17 subfamily A member 1; *ACSL3*: acyl-CoA synthetase long chain family member 3; *Nf2*: neurofibromin 2-like; *CYP11A1*: cytochrome P450 family 11 subfamily A member 1, ENSGALT00000099103, ENSGALT00000098365, MSTRG.18681.2, MSTRG.21492.1 and MSTRG.10917.2 are chicken LncRNA.

RESULTS

Transcriptome Sequencing Results

Analysis Sequencing data was compiled and quality assessments were performed, as shown in Table 2.

For the six samples from the two breeds, the range of Clean Reads obtained by sequencing was between 88,498,104 and 101,156,998. Each sample obtained at least 13,848,030,06 bases with a Q30 range of 93.12% to 93.92%. The N value was 0.00%, and the



GC content ranged from 48.03% to 54.07%, with an average GC content of 49.59%. Using Bowtie2 as the alignment tool, it was found that on average, 94.05% of the Mapped Reads data could be aligned with the chicken reference genome, with 90.27% of the data aligning with unique locations in the chicken reference genome. Overall, these results indicate good sequencing data quality.

lncRNA and mRNA characteristic analysis

On average, 16,546 mRNAs and 9,685 lncRNAs were identified from the six samples. The RNA sequence data were aligned with the chicken reference genome, known reads of lncRNA were removed, and the remaining data were analyzed using CPC2, CNCI, and

Feelnco to predict the coding potential of new transcripts. Transcripts without coding potential that intersected in the three software programs were considered reliable predictions, leading to the identification of 3,141 lncRNAs that were included in subsequent analyzes (Figure 1A). This set comprised 390 sense, 399 antisense, 84 intronic, 495 bidirectional, 9,989 intergenic, and 654 other lncRNAs (Figure 1B). Most lncRNAs had two, three, or four exons, significantly fewer than the number of exons found in mRNAs (Figure 1C). Although the length distribution range of lncRNAs and mRNAs was similar, lncRNAs tended to be longer than mRNAs (Figure 1D). Furthermore, the expression levels of lncRNAs were lower than those of mRNAs (Figure 1E).

Table 2 – Sequencing data assessment statistics.

Samples	Clean Reads	No. bases(bp)	Q30/%	N/%	GC/%	Reads/%
RC-1	92760010	13848003006	12951030294 (93.52%)	62603 (0.00%)	6684654362 (48.27%)	81857202 (90.45%)
RC-2	101156998	15050254130	14014730977 (93.12%)	67019 (0.00%)	8138027669 (54.07%)	80133439 (85.26%)
RC-3	98514568	14690282561	13735996659 (93.50%)	65976 (0.00%)	7396761618 (50.35%)	83855813 (89.76%)
TC-1	97567876	14540720656	13644185078 (93.83%)	66472 (0.00%)	7070509452 (48.63%)	86973831 (92.07%)
TC-2	88498104	14540720656	12327963879 (93.39%)	59967 (0.00%)	6365214603 (48.22%)	79152929 (91.91%)
TC-3	97799492	14540720656	13695439862 (93.92%)	66538 (0.00%)	7004013869 (48.03%)	87730452 (92.14%)

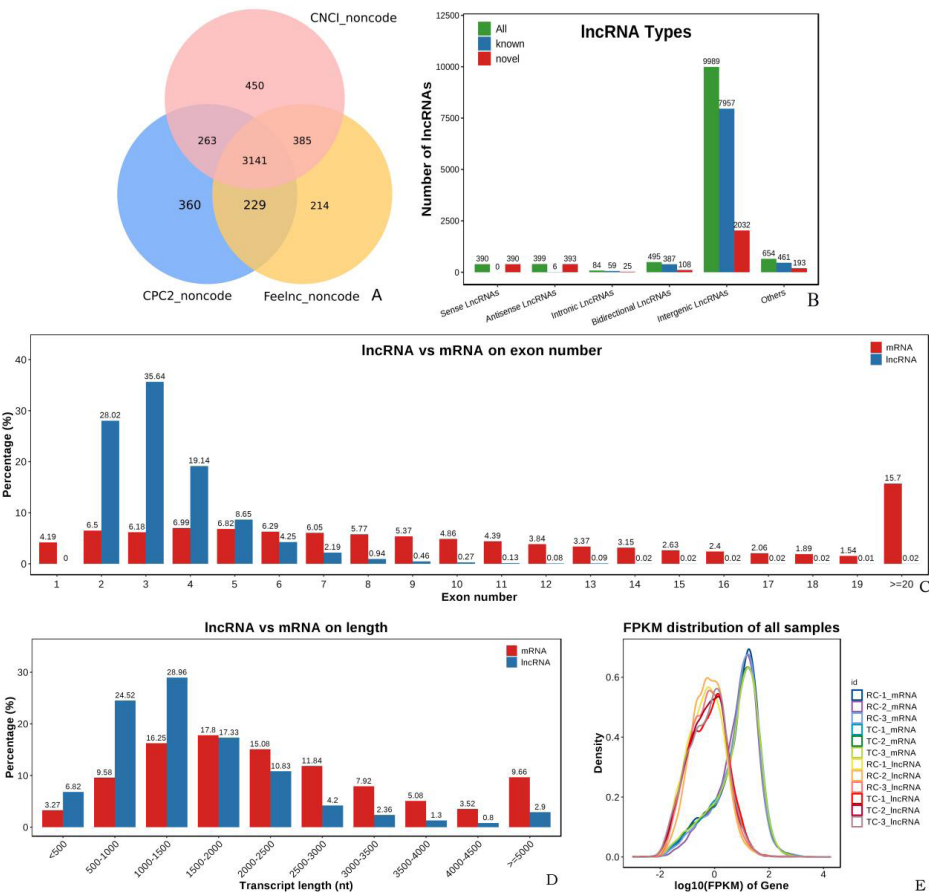


Figure 1 – Characteristics of lncRNAs and mRNAs in the ovarian tissues of the two breeds of chicken. (A) Screening of candidate lncRNAs by CNCI and CPC2. (B) Data on lncRNA transcript types. (C) Distribution of exon numbers in lncRNAs and mRNAs. (D) Length distribution of lncRNAs and mRNAs. (E) Expression levels of lncRNAs and mRNAs.



Analysis of differential expression and identification of lncRNAs and mRNAs

By setting filter criteria with $FDR < 0.05$ and $|\log_2 FC| > 1$, and a p -value < 0.05 for the comparison between the RC vs. TC groups, we identified 532 differentially expressed lncRNAs (DElncRNAs). Among these DElncRNAs, 240 were up-regulated and 292 were down-regulated (Figure 2A, C). We also identified a total of 2,314 genes significantly differentially expressed (DEmRNA) in the ovarian tissues of the RC

and TC groups, with 983 up-regulated, and 1,331 down-regulated genes (Figure 2B, D). When merging differentially expressed lncRNA and mRNA into a single group, both up-regulated and down-regulated lncRNA and mRNA showed good repeatability between groups in differential expression (Figure 2E, F).

Prediction of target genes for lncRNA and analysis of lncRNA-mRNA interactions

In the analysis of target genes, the predicted target genes of differentially expressed lncRNAs involved in cis

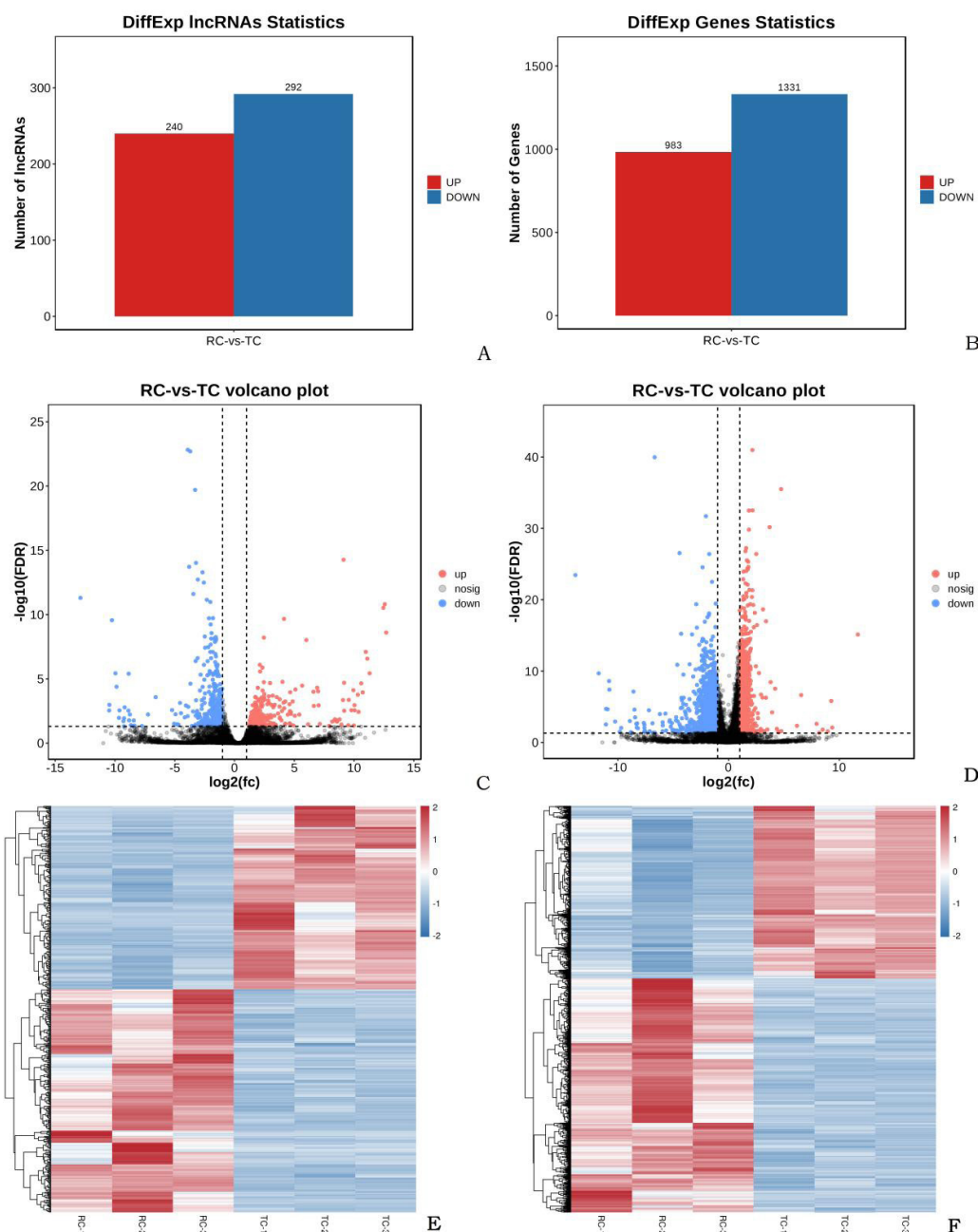


Figure 2 – Expression analysis of lncRNAs and mRNAs. (A) Numbers of differentially expressed up- and down-regulated lncRNAs. (B) Numbers of differentially expressed mRNAs (upregulated and downregulated). (C) Volcano plots displaying differentially expressed up- and down-regulated lncRNAs. (D) Volcano plots showing differentially expressed mRNAs. (E) Heatmap of differentially expressed lncRNAs. (F) Heatmap of differentially expressed mRNAs.



and transregulation were intersected with differentially expressed mRNAs. This intersection was used to analyze the potential genes present in Tibetan chicken ovarian tissue mRNAs that were influenced by the cis or trans-regulatory effects of lncRNAs. We discovered that differentially expressed lncRNAs targeted 48 differentially expressed mRNAs in cis regulation, and 14 in transregulation. Subsequently, an interaction network diagram was constructed for differentially expressed lncRNAs and their target genes of particular interest (Figure 3). Noteworthy interactions included differential expression of lncRNA MSTRG.21492.1 with ACSL3 and Nf2, MSTRG.18681.2 with PTPN5 and ACSL3, ENSGALT00000099103 with CYP11A1, MSTRG.10917.2, and ENSGALT00000098365 with CYP17A1 and ENSGALT00000098365 with ACSL3, indicating their interrelationships.

Differential expression prediction and functional analysis of target genes for lncRNA

To better understand the regulatory role of differentially expressed lncRNAs in the reproductive function of Tibetan chickens, we searched for protein-encoding genes within 10 kb upstream and downstream of the measured lncRNA genes. We predicted 43 differentially expressed lncRNAs and performed GO functional annotation, and KEGG functional clustering analysis on the significant target genes of these 43 lncRNAs. The GO functional annotation of all significantly differentially expressed lncRNA target genes revealed that these genes were annotated with 69 GO terms. Significant enrichment analysis of these GO terms revealed that

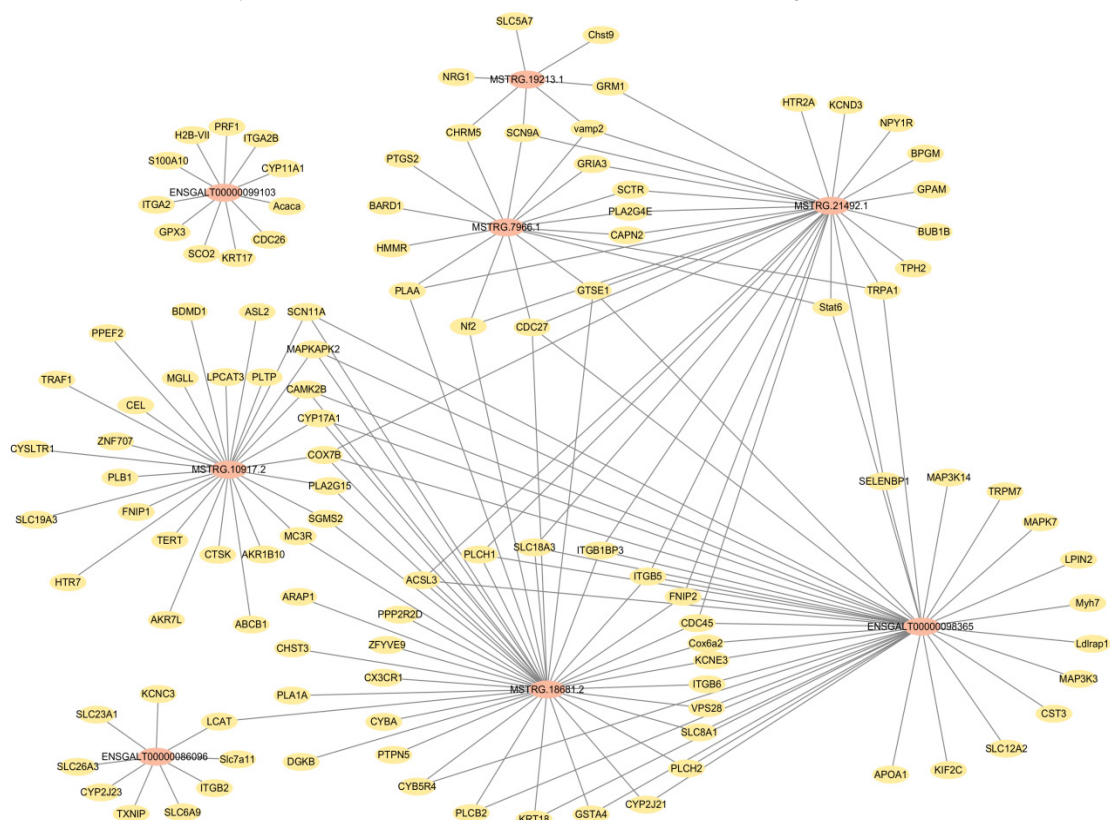


Figure 3 – Coexpression network of differentially expressed lncRNAs and differentially expressed mRNAs. Red represents lncRNA and yellow represents mRNA.

44 were significantly enriched ($p < 0.05$) (Figure 4A). These mainly included biological processes such as the presynaptic process in synaptic transmission (GO:0099531), rhythmic process (GO:0048511), behavior (GO:0007610), reproductive processes (GO:0022414), reproduction (GO: 000003), positive regulation of biological processes (GO:0048518), biological regulation (GO:0050789), signaling (GO: 0023052), development of cellular components,

tissues or organisms (GO: 0071840), metabolic processes (GO:0008152), among others. Furthermore, 13 cellular components and 8 molecular function terms were identified. The top 20 KEGG signaling pathways for lncRNA target genes are listed in Figure 4B, including pathways such as the TGF- β signaling pathway (ko04350), the metabolism of organic selenium compounds (ko00450), and oocyte meiosis (ko04114).

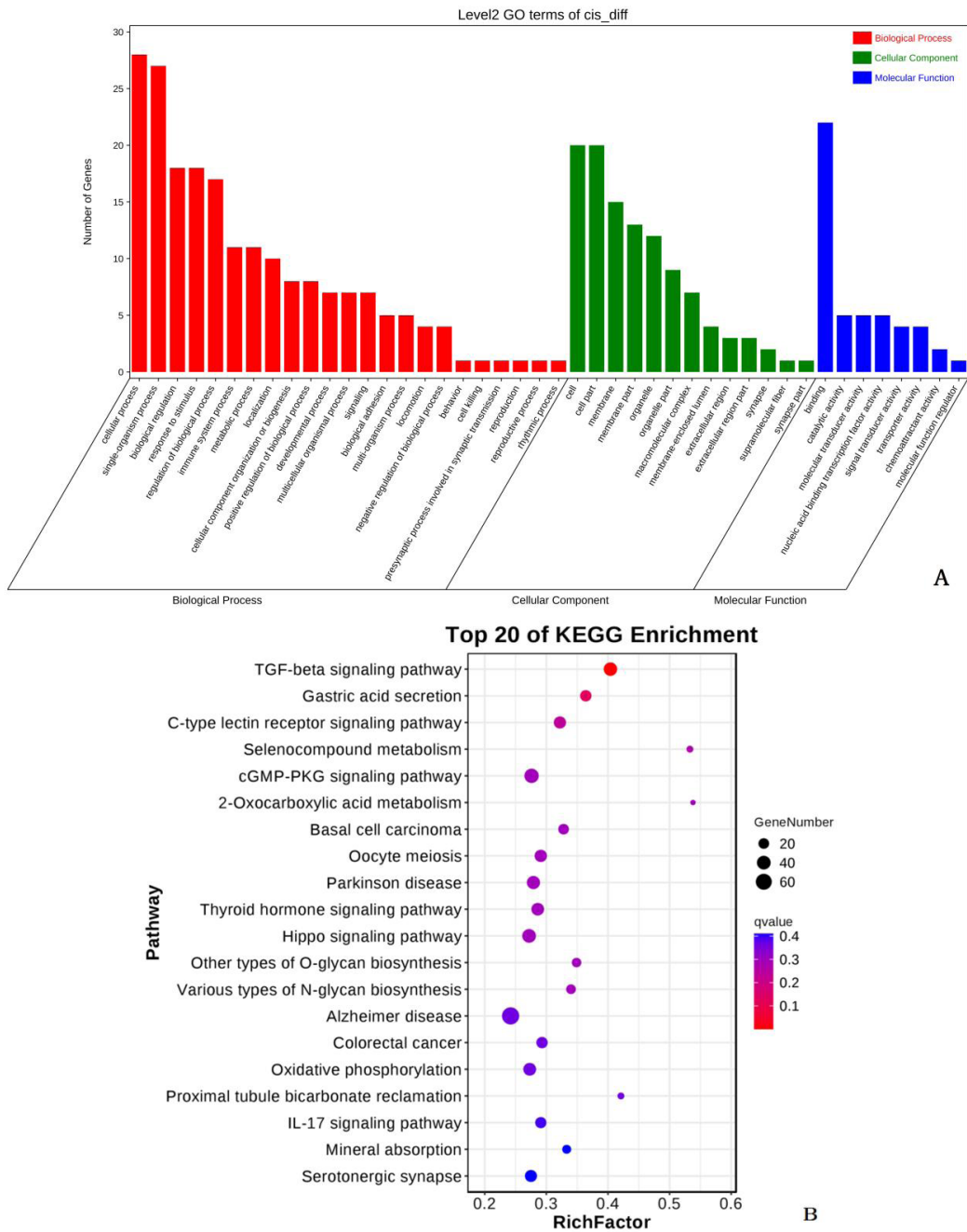


Figure 4 – GO and KEGG enrichment analyses of differentially expressed lncRNAs and mRNAs. (A) Annotated GO terms of target genes of differentially expressed lncRNAs. (B) Enriched KEGG pathways of target genes of differentially expressed lncRNAs.

Real-time fluorescent quantitative PCR validation

Real-time quantitative fluorescent PCR was used to validate five lncRNAs (Figure 5A). A comparison between the RC and TC groups revealed significant differences in MSTRG.10917.2, MSTRG.18681.2, ENSGALT00000099103, and ENSGALT00000098365 lncRNA ($p<0.05$). Furthermore, the three target genes (ACSL3, Nf2, and CYP11A1) showed significant differences between the two groups ($p<0.05$) (see Figure 5C). Comparing the results of real-time

quantitative fluorescent quantitative PCR amplification in real time with the transcriptome sequencing results (see Figure 5B, D) demonstrated similar expression trends, confirming the reliability of the sequencing results.

DISCUSSION

Tibetan chicken is a unique primitive local chicken breed from the Qinghai-Tibet Plateau of China, with a low degree of artificial selection. They have long-term

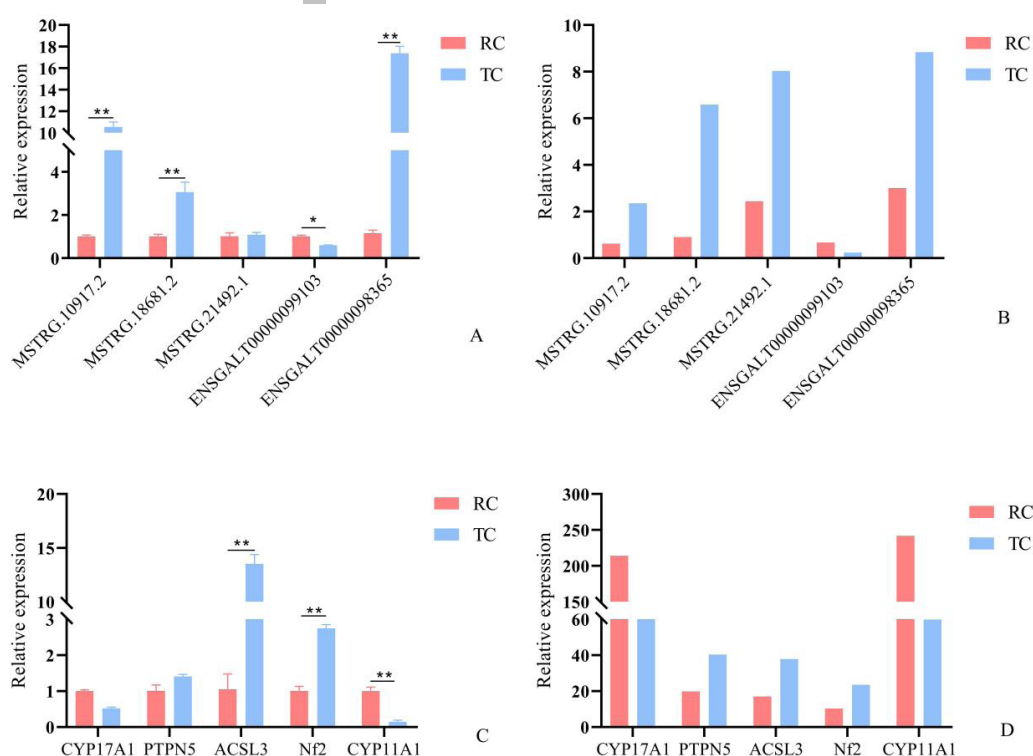


Figure 5 – Verification of differentially expressed lncRNAs and mRNAs by qRT-PCR. * indicates significant difference $p < 0.05$, ** indicates highly significant difference $p < 0.01$.

adaptation to high-altitude, low-oxygen environments, reach high levels of adaptability at the level of tissues, cells, and molecules, and possess a unique stable adaptive mechanism. Observations show that Tibetan hens start laying eggs around 240 days of age, with an average annual egg production of 40-80 eggs under grazing conditions, and some individuals lay more than 100 eggs. Tibetan chickens have always been popular among consumers. Although their relatively lower reproductive and growth performance prevents large-scale farming, the genetic mechanisms for high-altitude, low-oxygen adaptation in Tibetan chickens can be utilized to breed new high-yield varieties. In-depth research on the genetic mechanisms of egg production performance in Tibetan chickens can provide a theoretical basis for breeding improvement. Studying these animals can also help reveal their excellent genetic resources. Therefore, we analyzed the complex regulatory processes for egg production in Tibetan chickens at the transcriptome level to understand the factors that contribute to their low egg production. Screening genes related to regulating this trait, constructing a transcriptional expression regulatory network for low egg production in Tibetan chickens, and conducting GO and KEGG analyzes of differentially expressed genes revealed significant enrichment in reproduction, cellular composition, metabolic processes, and signaling pathways like

TGF- β and oocyte meiosis. Similar pathways have been selectively examined when studying the reproductive performance of other chicken breeds (Overbey *et al.*, 2021; Hanlon *et al.*, 2022; Xue *et al.*, 2021).

Excellent poultry productivity is measured by the efficiency of ovarian follicle development leading to ovulation or closure, as well as the efficiency of the oviduct in converting oocytes into shelled eggs (Hlokoe *et al.*, 2022). A well-organized follicle hierarchy is essential to improve egg production performance (Mfoundou *et al.*, 2021; Johnson. 2015; Li *et al.*, 2019)). Follicular development is a complex process, strictly regulated by various hormones and cytokines, including members of the transforming growth factor β (TGF- β) superfamily, with TGF- β playing a crucial role in ovarian function under physiological and pathological conditions (Knight *et al.*, 2003). Collagen secretion by granulosa cells (GC) in response to TGF- β 1 stimulation, and its transfer to adjacent theca cells (TC) promote cell proliferation, thus facilitating follicle development through a cooperative cellular model (Zhou *et al.*, 2021). The present study constructed a network map by screening 8 lncRNAs and their targeted mRNAs. These targeted mRNAs were found to be significantly enriched in various signaling pathways, including the TGF- β signaling pathway, organic selenium-containing compound metabolism, and oocyte meiosis. Importantly, these pathways have been implicated



in follicle development in chickens. Collectively, our findings suggest that the low laying performance observed in Tibetan chickens is not solely attributed to a single pathway, but rather involves coordinated expression changes of multiple distinct lncRNAs that trigger different target genes and metabolic pathways.

Differential gene expression related to follicle development, such as CYP11A1 and CYP17A1, was identified. These genes belong to the same gene family and promote ovarian growth and steroid hormone secretion (Sechman *et al.*, 2011; Zhang *et al.*, 2019; Wang *et al.*, 2023). Moreover, long noncoding RNAs (lncRNAs) targeting CYP11A1, and MSTRG.10917.2 and ENSGALT00000098365 targeting CYP17A1 suggest their regulatory roles in chicken reproductive traits. PTPN5 regulates gonadotropin-releasing hormone (GnRH)-induced follicle stimulating hormone (FSH) secretion through two parallel signaling pathways, Gs-protein kinase A (PKA) -PTPN5 and Gq-phospholipases C (PLC)-p38 MAPK-PTPN5, binding to GnRH and GnRH-r. PTPN5 also modulates gonadotropin function by regulating intracellular calcium homeostasis (Kulikova *et al.*, 2020; Wang *et al.*, 2021). This study found that PTPN5 is targeted by the lncRNA MSTRG.18681.2 in the ovaries of Tibetan chickens, potentially influencing their egg production performance. Vitellogenin (VTG), which is found primarily in the liver in a peptide form, undergoes chemical modifications such as phosphorylation and glycosylation, and circulates in the bloodstream until reaching the avian ovarian site, where oocytes absorb it via endocytosis (Li and Zhang, 2017). Within oocytes, VTG is broken down into vitellin and phospholipovitellin, gradually depositing to form the yolk. The synthesis of egg yolk in follicles involves two main pathways: endogenous synthesis by oocytes themselves and exogenous synthesis from outside oocytes, the latter being the primary source of yolk deposition (Schneider, 2009). In Tibetan chicken ovaries, VTG2 is targeted by the lncRNA MSTRG.22109.1. PLCB2 plays a significant role in the signaling pathways of GnRH, oxytocin, and estrogen (Yao *et al.*, 2021). GRIA3 is a neurotransmitter receptor located in the hypothalamic ionotropic α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), which is relevant to the secretion of FSH (Cole *et al.*, 2011). The results of this study suggest that the lncRNA ENSGALT00000099103 targets GRIA3 for molecular regulation in Tibetan chicken ovaries. Merlin, a product of Nf2, drives local estrogen formation, ultimately leading to granulosa cell differentiation and tissue repair (Macdougall *et*

al., 2001). ACSL3 inhibits lipid deposition and steroid synthesis in hierarchical follicles granulosa cells (hGCs) (Ran *et al.*, 2023). MSTRG.18681.2 promotes ACSL3, while MSTRG.21492.1 targets Nf2 and ACSL3 to regulate reproductive performance.

In summary, MSTRG.21492.1 targets ACSL3 and Nf2, while MSTRG.18681.2 targets PTPN5 and ACSL3; ENSGALT00000099103 specifically targets CYP11A1; both MSTRG.10917.2 and ENSGALT00000098365 target CYP17A1, and ENSGALT00000098365 targets ACSL3, thereby influencing the laying performance of Tibetan chickens through the regulation of follicle development and steroid hormone synthesis.

CONCLUSIONS

This study systematically identified the expression profiles of lncRNA and mRNA in the ovarian tissue of Tibetan chickens. Functional enrichment and interaction network analyzes suggest that the lncRNAs ENSGALT00000099103, ENSGALT00000098365, MSTRG.18681.2, MSTRG.21492.1, and MSTRG.10917.2, and the mRNAs PTPN5, CYP17A1, ACSL3, Nf2, and CYP11A1 may participate in the regulation of egg production performance in Tibetan chickens through processes such as follicle development and the secretion of reproductive hormones. These findings provide valuable resources for the breeding of high-altitude Tibetan chickens, and contribute to elucidating the molecular mechanisms underlying the regulation of reproductive performance in Tibetan chickens.

ACKNOWLEDGMENTS

The authors would like to thank Mengqi Duan for her helpful work during the internship.

Author contributions

Writing—original draft preparation, YRY and SK; writing—review and editing, FZL and YFG; supervision, ZYC; funding acquisition, YRY and ZYC. All authors have read and agreed to the published version of the manuscript.

Funding

This study was supported by the Joint Project of Northwest A&F University - Tibet Agricultural and Animal Husbandry College (XNLH2022-01) and the National Key Research and Development Program (2022YFD1600902-3).



Data availability statement

Data will be made available upon request.

Conflicts of interest

The authors declare no conflicts of interest.

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