Expression of Eleven Egg Performance-associated Genes in the Ovary of Zi Geese Anser anser domestica

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The relative expression of eleven egg performance-associated genes including the tau-crystallin/α enolase (ENO1), ribosomal protein S27a (RPS27A), calmodulin 1 (CALM1), solute carrier family 25 A6 (SLC25A6), aroylsulfatase A (ARSA), syndecan 2 (SDC2), ribosomal protein L10a (RPL10A), ornithine decarboxylase antizyme 1 (OAZ1), cytochrome c somatic variant 2 (CYCS), adenosine triphosphate synthase, H+ transporting, α subunit, isoform 1 (ATP5A1) and the ring finger protein 130 (RNF130) in the ovaries of pre-laying and laying Zi geese were analyzed using quantitative real-time PCR (qRT-PCR). Comparison between pre-laying and egg-laying stages, the relative expression of ENO1, RPS27A, SLC25A6, ARSA, SDC2, RPL10A, OAZ1, CYCS and RNF130 were significantly up-regulated (P<0.05), whereas the CALM1 and ATP5A1 were highly significantly up-regulated (P<0.01). This study established the primary foundation to understand the possible roles of these genes in the ovaries.

Key words: egg performance-associated genes, geese, ovaries, qRT-PCR


Introduction

Zi geese (Anser anser domestica) are excellent layers (around 100 per year) with a superior feed-to-egg conversion ratio (Kang et al., 2009). This species breeds in Northeast China (HeiLongjiang and JiLin provinces). In our previous study, in order to study the molecular genetic mechanisms of egg laying and improve laying performance, suppression subtractive hybridization (SSH) and reverse dot-blot were employed to identify egg laying performance-associated genes in the ovaries. We have discovered that the expression of 18 known and 8 unknown gene fragments were higher in the ovaries of egg-laying geese than pre-laying geese (Kang et al., 2009). In present study, tau-crystallin/α enolase (ENO1), ribosomal protein S27a (RPS27A), calmodulin 1 (CALM1), solute carrier family 25 A6 (SLC25A6), aroylsulfatase A (ARSA), syndecan 2 (SDC2), ribosomal protein L10a (RPL10A), ornithine decarboxylase antizyme 1 (OAZ1), cytochrome c somatic variant 2 (CYCS), adenosine triphosphate synthase, H+ transporting, α subunit, isoform 1 (ATP5A1) and the ring finger protein 130 (RNF130), which have been identified to be differentially expressed in the ovaries by SSH. Their expression were confirmed by using quantitative real-time PCR (qRT-PCR) in both egg-laying and pre-laying stages in the ovaries of the Zi geese. This results provided the primary foundation to understand the possible roles of these genes in egg formation in geese.

Materials and Methods

Geese and Tissue Collection

Twenty female Zi geese were selected on a local farm and were raised according to the program used in farm. All the geese were fed according to conventional farming (Kang et al., 2009). Ten geese were killed by exsanguination at 5-month-old (average BW=3.0±0.2 kg) and the ovaries were treated at pre-laying stage. Another 10 geese were killed at 8-month-old (average BW=3.5±0.2 kg) and the ovaries were treated at egg-laying stage. Ovaries samples, which comprised the whole ovary including the small and large yellow follicles, were rapidly removed, wrapped in foil, frozen in liquid nitrogen, and stored at −70°C until analysis.

Extraction of Total RNA and Quality Control

Total RNA was prepared by the Trizol reagent (Invitrogen Corporation, CA, U.S.A.) according to the instructions of the manufacturer. The total RNA samples from pre-laying and laying ovaries (n=10, for each) were pooled separately. Genomic DNA was removed by DNase 1 (Takara Biotech-
nology Company, Dalian, China) according to the instruction of manufacturer. Total RNA concentration and purity were determined by using a SmartSpec™ Plus spectrophotometer (Bio-Rad, CA, U.S.A.). Total RNA integrity was checked by electrophoresis on a denaturing agarose gel (Fig. 1a). All total RNA samples were stored at −70°C until analysis.

Reverse Transcription-PCR and qRT-PCR Analysis

In order to determine the expression of ENO1, RPS27A, CALM1, SLC25A6, ARSA, SDC2, RPL10A, OAZ1, CYCS, ATP5A1 and RNF130, first-strand cDNA was generated from 3μg total RNA pooled from pre-laying or laying goose ovaries (n=10) by using Superscript III (Invitrogen Corporation). Gene-specific primers were designed according to the cDNA sequences by using the Primer Premier 5.00 (Premier Biosoft International, CA, U.S.A.) and synthesized by Takara Biotechnology Company. The primers are listed in Table 1. In order to check the specificity of all primers further, PCR was performed on cDNA, and PCR products were analyzed on a 1% agarose gel (Fig. 1b). The qRT-PCR was performed in 50μL reactions on a Line-Gene K Real-Time PCR Detection System and software (Bioer Technology, Hangzhou, China), using 1μL of first-strand cDNA and SYBR Green PCR Master Mix (Takara Biotechnology Company). Thermal cycling was performed with an initial denaturation step of 10 s at 94°C, followed by 45 cycles of 5 s at 94°C, 56°C for 30 s, and then a final extension at 72°C for 20 s. Relative quantification of gene expression was performed in 3 replicates for each sample and normalized by β-actins (ACTB). As a control for genomic DNA contamination, an equivalent amount of total RNA without reverse transcription was tested for each sample per gene. A no-template control (NTC) was also included in each run for each gene. The quality of standard curves were judged by the slope of the standard curve and the square of the Pearson correlation coefficient (R²). The PCR amplification efficiency of each primer pair was calculated from the slope of a standard curve using the following equation: Efficiency % = (10^{−1/slope}−1)×100% (Bustin et al., 2009).

Statistical Analysis

The relative expression levels of ENO1, RPS27A, CALM1, SLC25A6, ARSA, SDC2, RPL10A, OAZ1, CYCS, ATP5A1 and RNF130 were calculated relative to ACTB (the normalizer) by using the comparative cycle threshold method (Livak and Schmittgen, 2001). The abundance of these genes in the ovaries of pre-laying geese were assigned a value of 1, and a Student’s t-test was performed to determine the differential significance of the genes by comparing the values from the ovaries of laying geese to those ovaries of

**Fig. 1.** Confirmation of amplicon size and primer specificity of studied genes. (a) Agarose gel electrophoresis showing the 28S and 18S rRNA bands, Lane (Ln) 1, total RNA sample of pre-laying Zi geese; Ln 2, total RNA sample of laying Zi geese; (b) Agarose gel electrophoresis showing specific reverse transcription PCR products of the expected size for each gene. Ln A, DL2000 DNA marker; Ln 1, ENO1; Ln 2, RPS27A; Ln 3, CALM1; Ln 4, SLC25A6; Ln 5, ARSA; Ln 6, SDC2; Ln 7, ACTB; Ln B, DL2000 DNA marker; Ln 8, RPL10A; Ln 9, OAZ1; Ln 10, CYCS; Ln 11, ATP5A1; Ln 12, RNF130; Ln 13, ACTB.
pre-laying geese. The correlation was subjected to analysis of variance (ANOVA) of the SAS software. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

Results

Only the samples with an A260/A280 ratio of 1.8–2.0 were chosen for analysis. Agarose gel electrophoresis (Fig. 1a) revealed that the 28S:18S ratio was approximately 2:1, which indicated that the samples are high quality RNA. Agarose gel electrophoresis (Fig. 1b) revealed that all primer pairs amplified a single PCR product with the expected size. The related information of the primers was listed in Table 2. The average value of the slope of the standard curve was in the range of $-3.6 \pm 0.5$ slope $\leq -3.1$, corresponding to an amplification efficiency of 90–110 %. The average value of $R^2$ was $\geq 0.98$. The qRT-PCR experiment was set up according to the MIQE guidelines (Bustin et al., 2009, 2010; Taylor et al., 2010).

The qRT-PCR results showed that ENO1, RPS27A, SLC25A6, ARSA, SDC2, RPL10A, OAZ1, CYCS, ATP5A1 and RNF130 were significantly up-regulated in the ovaries of laying geese compared with that of pre-laying geese ($P<0.05$), whereas CALM1 and ATP5A1 were highly significantly up-regulated in the ovaries of laying geese compared with that of pre-laying geese ($P<0.01$). The relative expression levels (Fig. 2) of ENO1, RPS27A, CALM1, SLC25A6, ARSA, SDC2, RPL10A, OAZ1, CYCS, ATP5A1 and RNF130 in the ovaries of laying geese increased by 2.34±0.67, 1.9±0.46, 4.31±0.18, 1.97±0.47, 1.98±0.26, 1.78±0.23, 10.36±1.83, 3.54±0.21, 4.35±0.36, 3.06±0.21, and 4.64±0.29 folds respectively compared with those of the pre-laying geese.

In addition, the correlation of mRNA expression was also analyzed between the eleven genes (Table 3). The correlation between ENO1 and RPS27A, CALM1 and ARSA were positive strong ($R^2 = 0.915, 0.818$ and $0.901; P<0.05$); The correlation between RPS27A and CALM1, ARSA and RPL10A were positive strong ($R^2 = 0.847, 0.974$ and $0.812; P<0.05$); The correlation between CALM1 and ARSA, RPL10A, OAZ1, CYCS, ATP5A1 and RNF130 were positive strong ($R^2 = 0.848, 0.930, 0.873, 0.916, 0.899$ and $0.911; P<0.05$); The correlation between SLC25A6 and ARSA, ATP5A1 were positive strong ($R^2 = 0.811, 0.841; P<0.05$); The correlation between SDC2 and CYCS were positive strong ($R^2 = 0.833; P<0.05$); The correlation between RPL10A and OAZ1, CYCS, ATP5A1 and RNF130 were positive strong ($R^2 = 0.987, 0.986, 0.985$ and $0.886; P<0.05$); The correlation between OAZ1 and CYCS, ATP5A1 and RNF130 were positive strong ($R^2 = 0.956, 0.924; P<0.05$); The correlation between ATP5A1 and RNF130 was positive strong ($R^2 = 0.866; P<0.05$).

Discussion

Analysis of mRNA transcription in the ovaries is a very important method to find the relationship between gene function and egg-production (Yen et al., 2006; Chen et al.,
laying geese were higher than pre-laying geese (Yen et al., 2009). This paper described the relative expression profiles of eleven egg-performance associated genes in ovaries of both pre-laying and laying Zi geese.

ENO1 is a key enzyme in the glycolytic pathway which catalyzes the reversible conversion of D-2-phosphoglycerate into phosphoenol-pyruvate in the second half of the glycolytic pathway (Avilán et al., 2011). Recent studies have revealed that ENO1 can be detected in many prokaryotic and eukaryotic cells, and localized in cytoplasm, cell surface and nucleus of various mammalian cells to possibly mediate distinct functions (Liu et al., 2007). ENO1 gene up-regulation in mRNA and/or protein levels have been observed in several tumors, including brain, breast, cervix, colon, eye, and so on (Liu et al., 2007; Capello et al., 2011).

ENO1 is associated with tumor development through a process known as the Warburg effect. Tumor tissues or other normal proliferative tissues consume more glucose by the pathway of glycolytic than normal cells and generate ATP by converting pyruvate to lactic acid, even in the presence of a normal oxygen supply (Vander et al., 2009). This study found that the relative expression levels of ENO1 in the ovaries of laying geese compared with those of pre-laying geese were increased by 2.34 ± 0.67 fold. It has been found that the mRNA concentrations of ENO1 in the livers of laying geese were higher than pre-laying geese (Yen et al., 2009). We propose that the rapid growth and development of avian ovarian follicle also need consume glucose by the pathway of glycolytic as the dynamics of avian ovarian follicle development. Futher studies are needed to determine the role of ENO1 in avian ovarian follicle growth and development.

Ribosomal protein is an essential component of the 40S subunit of the ribosome, and it was proved that it has an intimate relationship with protein synthesis (Shang et al., 2005). Follicular morphous and function of the poultry in breeding season generate several changes, which indicated that the large amounts of proteins were synthesized not only to activate the maturation promoting factor (MPF) but also have a close relationship with the developmental capacity of postfertilization megagametocyte. *Mus musculus* ribosomal protein gene S29 and human follicular cell ribosomal protein gene S25 and S18 conspicuous differential expression in different stages of mammal follicular development (Dharma et al., 2009). RPS27A was also found to be up-regulated in matured buffalo (*Bubalus bubalis*) oocytes (Kandil et al., 2010). The up-regulation of S27A in laying season in our research shows that it may be involved in the protein synthesis during the follicle alveolar substance maturation process.

CALM1, which is a ubiquitous calcium-binding protein and mediator of the calcium signal, is known to regulate numerous cellular processes, including cell motility, proliferation, and intermediary metabolism (Toutenhoofd et al., 1998). CALM1 can activate the Ca\(^{2+}\)-transporting system in the avian shell gland for maintenance eggshell formation (Lundholm et al., 1990). CALM1 is also critical for up-regulation of steroidogenesis in the theca cells of hens (Levorse et al., 1991). In addition, CALM1 was highly expressed in the ovary of laying geese, which implies that CALM1 may be involved in the process of egg laying in geese.

SLC25A6, which was expressed at high levels in the ovaries of laying geese, is localized to the inner mitochondrial membrane (Palmieri, 2004); these proteins were referred as mitochondrial transporter family. SLC25A6 consists of proteins that function as transporters of a large variety of molecules (i.e. ATP, ADP, citrate, malate, Ornithine, citrulline, lysine, arginine, histidine, coenzyme A) (Fiermonte et al., 2009). Therefore, the up-regulation of SLC25A6 may be the result of high metabolic activity in the ovaries (Kang et al., 2009).

*ARSA* is involved in hydrolase activity, catalytic activity, and cerebrosidesulfatase activity. *ARSA* almost located everywhere in organism, for example, *ARSA* exist in cytososome in somatic cell as lysosomal enzyme, butin the perforatorium as acrosomal enzyme and cytolemma surface of spermatooza and ovum as membrane peripheral protein. *ARSA* keeps high conservation organic evolution and exist in almost animals including mammalia, fish and birds. The research showed that *ARSA* unfertilized only bind with ovum...
but not with spermatovum (Wu, 2004), which suggested that the close correlation of ARSA and sperm-egg fusion may affect the egg laying. It’s up-regulation in the ovary of laying geese suggests an association with laying performance (Kang et al., 2009).

The syndecans are a family of cell-surface heparan sulfate proteoglycans that regulate cell behavior through the binding of extracellular matrix molecules and/or soluble ligands (Zimmermann and David, 1999). This interaction regulates cell-ECM adhesion, migration, cytoskeleton organization, and gene expression as the membrane protein of receptor through signal transduction pathways (Park et al., 2002). It is concluded that the up-regulation of SDC2 in ovaries of laying Zi geese may affect follicular proliferation by regulating follicular maturation.

The OAZ1 inhibits ornithine decarboxylase (ODC) and accelerates its degradation (Matsufuji et al., 1995). ODC is a key enzyme in the biosynthesis of the polyamines and its activity is regulated in response to factors that stimulate cell proliferation (Schipper et al., 2000). Steady-state ODC mRNA levels and enzyme activity were measured in tissues of chicks genetically selected for increased growth rate or for

![Expression of eleven egg performance-associated genes in the ovary of Zi geese.](image-url)

Fig. 2. Expression of eleven egg performance-associated genes in the ovary of Zi geese. Expression of ENO1, RPS27A, CALM1, SLC25A6, ARSA, SDC2, RPL10A, OAZ1, CYCS, ATP5A1 and RNF130 genes in ovaries of pre-laying geese (prelay) compared with those of laying geese (lay). The expression of these genes were normalized to ACTB. Gene expression levels, calculated by the Δ-Δ threshold crossing point method, are presented in arbitrary units (AU). * Significantly different ($P < 0.05$) from prelay; ** highly significantly different ($P < 0.01$) from prelay. Values are means ± SD ($n = 10$).
egg production (Johnson et al., 1995). Carmen et al. (2002) indicated that although elevated levels of ODC are not required for acute ovarian steroidogenesis, the preovulatory peak of ovarian ODC activity observed in the evening of proestrus may be critical for the establishment of a constitutive steroidogenic pathway and progesterone secretion by the corpus luteum during the diestrus stage of the murine estrous cycle. The up-regulation of the corpus luteum during the diestrus stage of the murine

tutive steroidogenic pathway and progesterone secretion by proestrus maybe critical for the establishment of a consti-

laying Zi geese may be the result of the increase of OAZ1 maybe hy pothesized that

and its absence results in cell death (Baran et al., 2007). Therefore, the expression level of ATP5A1 was higher in the oocytes of laying geese because of a great quantity of ATP generated by aerobic respiration.

Ring finger proteins could play an important role in energy metabolism, protein synthesis, oocyte maturation, tumorigen-

esis (Fang et al., 2003; Hirvonen-Santti et al., 2004; Koyama et al., 2008). In present study, the relative expression level of RNF 130 in the ovaries of laying geese increased by 4.64 ±0.29 fold compared with those of pre-laying geese. It may be the result of high metabolic activity in the ovaries.

The correlation results suggested that there are certain synergistic effect among some proteins encoded by the above certain genes (positive strong correlation) in the mature process of follicle. For example, CALM1 could regulate numerous cellular processes, and accordingly have strongly correlated with great majority of genes. In addition, some genes were closely associated with others such as RPL10A and OAZ1, CYCS, ATP5A1, RNF130, because they maybe

Table 3. Analysis of the eleven genes correlation

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<th>RPL10A</th>
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* P<0.05; ** P<0.01

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gestions.
References


Zimmermann P and David G. The syndecans, tuners of transmembrane signaling. FASEB Journal, 13 (Suppl.), S91-S100. 1999.