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Divergently expressed gene identification and interaction prediction of long noncoding RNA and mRNA involved in duck reproduction

Jindong Ren[a,b], Xue Du[b], Tao Zeng[b], Li Chen[b], Junda Shen[b], Lizhi Lu[b,*], Jianhong Hu[a,*]

[a] College of Animal Science and Technology, Northwest A & F University, No. 21 Xinong Road, Yangling, Shaanxi 712100, PR China
[b] Zhejiang Academy of Agricultural Sciences, No. 198 Shiqiao Road, Hangzhou, Zhejiang 310021, PR China

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ABSTRACT

Long noncoding RNAs (lncRNAs) and divergently expressed genes exist widely in different tissues of mammals and birds, in which they are involved in various biological processes. However, there is limited information on their role in the regulation of normal biological processes during differentiation, development, and reproduction in birds. In this study, whole transcriptome strand-specific RNA sequencing of the ovary from young ducks (60 days), first-laying ducks (160 days), and old ducks, i.e., ducks that stopped laying eggs (490 days) was performed. The lncRNAs and mRNAs from these ducks were systematically analyzed and identified by duck genome sequencing in the three study groups. The transcriptome from the duck ovary comprised 15,011 protein-coding genes and 2905 lncRNAs; all the lncRNAs were identified as novel long noncoding transcripts. The comparison of transcriptome data from different study groups identified 2240 divergent transcription genes and 135 divergently expressed lncRNAs, which differed among the groups; most of them were significantly downregulated with age. Among the divergent genes, 38 genes were related to the reproductive process and 6 genes were upregulated. Further prediction analysis revealed that 52 lncRNAs were closely correlated with divergent reproductive mRNAs. More importantly, 6 remarkable lncRNAs were correlated significantly with the conversion of the ovary in different phases. Our results aid in the understanding of the divergent transcriptome of duck ovary in different phases and the underlying mechanisms that drive the specificity of protein-coding genes and lncRNAs in duck ovary.

1. Introduction

Ducks are one of the most economically important domestic poultry species, as they are a source of meat, eggs, and feathers. According to FAO 2010, duck meat is vital to the livestock meat production worldwide at approximately 2% of the total livestock production, and duck meat consumption has increased in recent years (Ibrahim and Lamidi, 2014). Additionally, compared to chicken eggs, duck eggs are considered of higher quality, because they contain less water and higher percentage of proteins and fats in the yolk (Biosci et al., 2015). Ducks diverged from the related chicken and turkey at the genome level (Hackett et al., 2008; Huang et al., 2014), and they differ in reproductive performance (Zheng et al., 1989). The complete duck genome (Huang et al., 2014)

* Corresponding authors.
E-mail addresses: renjindong@126.com (J. Ren), sd_duxue@126.com (X. Du), zengtao4009@126.com (T. Zeng), kuaileqianjin@163.com (L. Chen), shenjunda66@sina.com (J. Shen), lulizhibox@163.com (L. Lu), hjh19732008@126.com (J. Hu).

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provides detailed information and insight into the regulatory mechanisms of reproduction. The diversity found in poultry renders ducks particularly well suited for studies on the regulatory mechanism of reproduction.

The long non-coding RNA (lncRNA) is a non-translated RNA longer than 200 bp; this RNA was defined for the first time and its function described systematically by Spizzo et al. (2012). Since then, the abundance of novel lncRNAs has been documented and their role in various critical biological process was confirmed, such as in cell proliferation, differentiation, chromatin remodeling, epigenetic regulation, genomic slicing, transcription, and translation (Ponting et al., 2009; Chen, 2015). Specifically, the genetic mechanisms of dosage compensation, epigenetics, cell cycle regulation, and cellular differentiation implicate inhibition of proteins by binding of lncRNA to proteins or to miRNAs or by titration of miRNAs (Xiao et al., 2009; Wapinski and Chang, 2011). LncRNAs can serve as signals, decoys, guides, and scaffolds, according to their molecular mechanism (Wang and Chang, 2011). LncRNAs also have a very complex regulation network, such as that of the *H19* gene (Monnier et al., 2013), but most of the mechanisms of lncRNA-related regulation in biological processes remain unclear. With the development of new technologies for lncRNA identification, biological functions of lncRNAs, such as development of different diseases (Chen, 2015; McHugh et al., 2015), have been receiving increasing attention in research. Studies on liver cancer, lung cancer, and other diseases demonstrated that the expression level of some specific lncRNAs is associated with progression of these cancers (Hrdlickova et al., 2014; Li et al., 2014), rendering them an effective biomarker in diagnosis of various types of cancers (Ariel et al., 2000; Maass et al., 2014). In genetics, the effects of lncRNAs on important phenotypic characters have been researched (Frésard et al., 2014; Li et al., 2015), especially for their possible application in breeding suggested by poultry breeding organization (Fulton, 2012). Moreover, there is very limited information about lncRNAs in poultry (Arriaga-Canon et al., 2014), particularly domestic poultry such as duck and goose. Therefore, research on divergent transcription of long noncoding RNA in reproduction will contribute to our knowledge about lncRNAs and identify potential biomarkers for duck breeding.

The most intensively studied and best understood lncRNA related to reproduction is *Xist,* which is involved in X chromosome inactivation, while *Xist* itself is regulated by histone methylation, thereby affecting reproduction via related genes in humans (Plath et al., 2002; Ponting et al., 2009; Lee, 2010; Sai and Chandra, 2016). LncRNAs also play an important role in the lifecycle and reproduction (Hung et al., 2011). LncRNAs as epigenetic markers contribute to determination of reproduction-related genes in sex chromosome silencing or active transcription and participate in lineage- and tissue-specific expression of genes in birds (Frésard et al., 2014). Therefore, the aim of the present study was to identify novel lncRNAs and effective biomarkers for reproductive breeding of ducks (*Anas platyrhynchos*).

2. Materials and methods

2.1. Tissue sampling and RNA preparation

The experimental duck care and slaughter protocol was approved by the Institutional Animal Care and Use Committee of Northwest Agriculture and Forestry University, and performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals. All treatments were performed, and all efforts were made to minimize suffering of the experimental ducks. The ducks were treated equally before sample collection. All ovary tissue samples were collected at the Zhongwang Beijing Duck Breeding Farm (Huzhou, China). Three birds from each of the three different age groups (young ducks [YD; 60 days old], first-laying ducks [FL; 160 days old], and old ducks [OD; 490 days old], i.e., ducks that stopped laying eggs) were slaughtered for tissue sampling. Fresh tissue samples were washed in PBS (Gibco, Fisher Scientific, Waltham, MA, USA) and instantly frozen in liquid nitrogen. Total RNA was extracted from each sample using an Agilent 2100 RNA Nano 6000 Assay Kit (Agilent Technologies, Santa Clara, CA, USA). RNA concentration and purity were determined with a spectrophotometer (NanoVue; GE Healthcare, Piscataway, NJ, USA).

2.2. RNA-seq and transcript identification

A total amount of 3 μg RNA per sample was used as initial material for RNA sample preparation. Ribosomal RNA was extracted using an Epicentre Ribo-Zero™ Gold Kit (Epicentre Technologies, Madison, WI, USA) and employed to generate sequencing libraries with varied index label with a NEBNextUltra™ Directional RNA Library Prep Kit for Illumina (New England BioLabs, Ipswich, MA, USA) following manufacturer's recommendations. The clustering of the index-coded samples was performed on a cBot cluster generation system using a TruSeq PE Cluster Kit v3-cBot-HS (Illumina, San Diego, CA, USA), according to manufacturer's instructions. After cluster generation, the libraries were sequenced on an Illumina Hiseq 4000 platform and 150 bp paired-end reads were generated. The clean data were obtained by filtering the raw reads and removing polluted reads, low quality reads, and reads with unknown bases accounting for more than 5%. Filtered reads were aligned to the duck genome by using TopHat (version 2.0.12) (Trapnell et al., 2009) and mapped with Bowtie2 (version 2.2.3) (Langmead et al., 2009).

Novel transcripts and novel lncRNAs were identified by aligning the new transcriptome and the reference genome using Cuffcompare (version 2.2.1) and filtering the transcriptome with the character lncRNAs (Moran et al., 2012). The coding potential of each transcript was predicted by CPC (Kong et al., 2007) and CNCI (Sun et al., 2013) to eliminate protein-coding transcripts.

2.3. Quantitation of gene expression levels

The number of all clean reads for each gene in each sample was counted with HTSeq (version 0.6.0) (Anders et al., 2015), and