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Research paper

Transcriptome analysis of the mammary gland from GH transgenic goats during involution

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ABSTRACT

Mammary glands are organs for milk production in female mammals. Growth hormone (GH) is known to affect the growth and development of the mammary gland, as well as to increase milk production in dairy goats. This study performed a comprehensive expression profiling of genes expressed in the mammary gland of early involution GH transgenic (n = 4) and non-transgenic goats (n = 4) by RNA sequencing. RNA was extracted from mammary gland tissues collected at day 3 of involution. Gene expression analysis was conducted by Illumina RNA sequencing and sequence reads were assembled and analyzed using TopHat. FPKM (fragments per kilobase of exon per million) values were analyzed for differentially expressed genes using the Cufflinks package. Gene ontology analysis of differentially expressed genes was categorized using agriGO, while KEGG pathway analysis was performed with the online KEGG automatic annotation server. Our results revealed that 75% of NCBI goat annotated genes were expressed during early involution. A total of 18,323 genes were expressed during early involution in GH transgenic goats, compared with 18,196 expressed genes during early involution of non-transgenic goats. In these expressed genes, the majority (17,589) were ubiquitously expressed in GH transgenic and non-transgenic goats. However, there were 745 differentially expressed genes, 421 of which were upregulated and 324 were downregulated in GH transgenic goats. GO and KEGG pathway analysis showed that these genes were involved in mammary gland physiology, including cell adhesion molecules, ECM-receptor interaction, Jak-STAT signaling pathway, and fat metabolism. Our results demonstrated that the GH receptor was strongly affected in GH transgenic goats, which may activate the IGF-1/Stat3 signaling pathway. Overall, our study provided a global view of the transcriptome during involution of GH transgenic and non-transgenic goats, which increases our understanding of the biology of involution in the goat.

1. Introduction

The milk of goats is similar to human milk, and low milk production can hinder the development of dairy goats. Milk yield is dependent on the number and metabolic activity of secretory cells in livestock (Boutinaud et al., 2004). Accordingly, lactation performance may be improved by increasing mammary cell proliferation or decreasing apoptosis of secretory cells. Furthermore, factors involved in the regulation of these processes can directly impact mammary function and milk yield. One of the primary factors affecting mammary gland function is hormones, including growth hormone (GH), prolactin, estrogen, and insulin (Shingu et al., 2004; Zarzynska and Motyl, 2005). GH, secreted from the pituitary gland, is widely known for its galactopoietic effect in lactating dairy animals (Eppard et al., 1985). A positive relationship between GH and milk production has been observed (Mullen et al., 2011). GH can increase the synthesis of milk protein, but can also stimulate the differentiation and proliferation of mammary gland epithelial cells (Zhou et al., 2008; Johnson et al., 2010). Although milk production can be improved by the administration of GH in livestock (Molik et al., 2010), the role of GH in goat lactogenesis remains unclear.

In our previous study, we constructed a mammary gland-specific vector expressing GH in the mammary during lactation and produced transgenic dairy goats harboring the GH gene using SCNT (somatic cell nuclear transfer) (Zhang et al., 2014a). In prenatals, the udders of GH transgenic goats were larger than non-transgenic goat udders. During

Abbreviations: GH, growth hormone; RNA, ribonucleic acid; FPKM, fragments per kilobase of transcript per million mapped reads; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; NCBI, National Center for Biotechnology Information; ECM-receptor, extracellular matrix receptor; IGF-1, insulin-like growth factor 1; Elf5, E74-like factor 5; RNA-seq, RNA sequencing; SCNT, somatic-cell nuclear transfer; TG, GH-transgenic goats; NG, non-transgenic goats; cDNA, complementary DNA; PCR, polymerase chain reaction; EB, ethidium bromide; qRT-PCR, quantitative real-time polymerase chain reaction; ACTB, beta-actin; ZBTB7B, zinc finger and BTB domain-containing protein 7B; GLYCAM1, glycosylation-dependent cell adhesion molecule -1; GHR, growth hormone receptor; CSN1S2, alpha-S1-casein; LALBA, lactalbumin, alpha; GHITM, growth hormone-induced transmembrane protein; FTH1, ferritin heavy chain; SPP1, secreted phosphoprotein 1; LGB, β-lactoglobulin; IGFBP-5, insulin-like growth factor-binding protein 5; STAT3, signal transducer and activator of transcription 3.

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Table 1
qRT-PCR primers used in evaluating the RNA-seq data.

Gene	Sense primer	Anti-sense primer	Products
CSN2	tgaaaagctgcaccttcctc	tctggggcactactttctgg	152 bp
CSN3	gcccaaactcttcaatggc	cggtggtaggtgtactgtgt	204 bp
CSN1S2	tactcccaccgtgaacagag	aggttgagtccatggcttca	231 bp
LALBA	tttggtgcaaagacgaccag	acagagtgctttatgggcca	157 bp
GLYCAM1	agctgcctctgtccatactc	cttcagaggcaggagggaat	203 bp
GHR	tgtccatacacagctcagca	gcgctgtccatgatgaagtt	188 bp
SPP1	gatggccgaggtgatagtgt	ggaaagctcgtcactgttgg	194 bp
FTH1	cgctgtgcttggaaagaagt	aggcttagctgtcactgtgt	239 bp
Elf5	ttgtgcgagacctgcttcta	cccgttccaaaattccggtt	200 bp
GHITM	gtggcccctctgacgatatt	ccaccatagactgccactga	238 bp
SPARC	catcggcgagtttgagaagg	ttgttgtcctcgtcccctctc	245 bp
Stat3	agcagcaaagaaggaggagt	ttccgaatgcctcctccttt	216 bp
IGFBP-5	tgagacaggaatccgagcag	catacttgtccacgcaccag	194 bp
IGF-1R	cactcactctgacgtctggt	ccttgacgctgctgatgatc	232 bp
Leptin	tctaccaacagatcctcgcc	gtggagtagagggaggcttc	183 bp
ATCB	ccactggcattgtcatggac	tagccatctcctgctcgaag	242 bp

the lactation period, the milk production of GH transgenic goats was higher than in non-transgenic goats. During dry periods, despite GH showing no expression in the goat mammary, the mechanism affecting involution of the mammary in GH transgenic goats remains unclear. Since the mechanism behind lactation is a complex regulatory network, a more detailed study on the regulatory mechanism is required.

Recently, advances in methodologies such as high-throughput sequencing technologies have allowed for the investigation of transcriptomes in mammary glands during pregnancy, lactation, and dry periods (Bionaz et al., 2012; Wickramasinghe et al., 2012). Transcriptome analyses have been conducted in bovines to uncover mammary gland adaptations during the lactation period (Cui et al., 2014), but the involution period of goats has not been examined. Herein, we used Illumina/Solexa deep-sequencing technology to identify and compare the full repertoire of gene expression in mammary glands of GH transgenic and non-transgenic goats during the involution period. Our results provide valuable information on the differences in gene expression and regulation during lactation between GH transgenic and non-transgenic goats.

2. Materials and methods

2.1. Tissue collection and RNA isolation

Eight mammary gland tissues of Sannen goats were obtained from GH-transgenic goats (TG) (n = 4) and non-transgenic goats (NG) (n = 4) at the Research Farm of Shanghai Transgenic Research Center (Nanhui, Shanghai, China). GH transgenic goats were generated by somatic cell nuclear transfer (SCNT) with SalI/PvuI-linearized plasmid pcGH, which contained 2.3 kb of the goat β-lactoglobulin proximal promoter region, the coding region of goat GH gene and 2.1 kb of the 3′β-lactoglobulin region. The NG and TG goats were all 3 years old and seeded in the same condition in Shanghai. All goat used in this experiment were cultured in the same condition. These goats' mammary gland development and lactation period were in the same time. All trials were conducted in accordance with the Guidelines for the Care and Use of College of Veterinary, Nanjing Agricultural University and Shanghai Transgenic Research. Mammary tissue was harvested and immediately frozen in liquid N2 and preserved at −80 °C. Total RNA was extracted from the mammary gland of eight Saanen goats with Trizol reagent (Invitrogen, USA). Quantity and purity of isolated RNA samples were analyzed using Agilent Bioanalyzer 2100 (Agilent, CA) before generation of sequencing libraries.

Table 3
Distribution of expression values of the differentially expressed genes.

FPKM range	NG (frequency)	TG (frequency)
(0, 10)	22889	22897
(10, 20)	495	478
(20, 30)	198	218
(30, 40)	108	112
(40, 50)	73	57
(50, 60)	40	25
(60, 70)	23	34
(70, 80)	27	32
(80, 90)	24	17
(90, 100)	18	16
(100, INF)	274	283

TG: mammary gland of GH transgenic goat in involution period.
NG: mammary gland of non-transgenic goat in involution period.

2.2. RNA sequencing

Isolated polyadenylated RNA was selected with oligo (dT) bead and subjected to Illumina's RNA-Seq library generation. Firstly, fragmentation buffer was added for turning RNA into short fragments. Taking these short fragments as templates, a random primer was exploited to generate the first-strand cDNA. Subsequently, the second-strand cDNA was synthesized. Secondly, a paired-end library was established with the genomic sample prep kit (Illumina, San Diego, CA) according to the manufacturer's instructions. Short fragments were purified with QIAquick PCR extraction kit and resolved with EB buffer for end repair and adding polyA. After that, the short fragments were connected with sequencing adapters. For amplification with PCR, we selected suitable fragments as templates, with respect to the result of agarose gel electrophoresis. At last, the library was sequenced using Illumina seq2000. The goat reference genome sequences were downloaded from NCBI. After removing the low quality read and clipping the adapter sequences, the clean reads from each sample were aligned to the goat reference genome using TopHat (Trapnell et al., 2009), a gapped aligner capable of discovery splice junctions ab initio. TopHat reports aligned results from the two mapping steps in shoot apical meristem format for further analysis. Aligned reads from TopHat mapping were subjected to Cufflinks (Trapnell et al., 2010). The gene expression was estimated using Cufflinks and normalized by calculating the reads per kilo base per million mapped reads (FPKM) for each gene and annotated with NCBI genome assembly (Mortazavi et al., 2008).

2.3. Gene ontogeny (GO) categorization

Differentially expressed genes between non-transgenic goat group and GH transgenic goat group were chosen to perform gene ontology analysis with agriGO (Du et al., 2010), an automated tool for the assignment of GO terms. The annotation result was categorized with respect to biological process, molecular function, and cellular component.

Table 2
RNA sequencing statistics for the TG and NG libraries.

Sample	Clean reads	Mapped reads	Mapped rate	Mapped gene number	Total gene number	Mapped gene rate
NG	61856468	38020630	61.46%	18198	24167	75.30%
TG	50428696	40052628	79.42%	18325	24167	75.82%

TG: mammary gland of GH transgenic goat in involution period.
NG: mammary gland of non-transgenic goat in involution period.