Transgenerational effects on the gene transcriptome of chicken liver

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Abstract

Chickens are important breeding animals and models for biomedical research, particularly due to their oviparous nature, which makes it an ideal subject for studying maternal effects. This study employs RNA-Seq to conduct a comprehensive analysis of the transcriptomics of the poultry liver, with a focus on maternal transgenerational effects. Samples were examined from broiler breeders, E19 embryos, and 21-day-old offspring, identifying 2,753 DEGs. GO analysis revealed significant enrichment of differentially expressed RNAs in functions such as actin filament binding and lysosomal activity. KEGG analysis identified pathways associated with endocytosis and Toll-like receptor signaling, displaying a high-low-high expression pattern across the broiler breeders, embryos, and offspring, which is closely linked to immune function regulation. Conversely, the Neuroactive ligand-receptor interaction and Calcium signaling exhibited a low-high-low expression pattern, which is intimately associated with organogenesis, and embryonic development. Additionally, based on DEGs, genes such as *IGF1*, *IGFBP*, *FASN*, and *ELOVL* were identified, which are significantly expressed in embryos and are crucial for development and lipid metabolism regulation. In summary, the present research provides a valuable transcriptional regulatory network for studying maternal effects on liver tissue development in broiler breeders, laying a foundation for further exploration of the molecular mechanisms underlying maternal effects.

Introduction

Maternal transgenerational effects significantly influence the phenotypic traits of the offspring, encompassing factors such as nutrition, hormones, gut microbiota, and epigenetic regulation[1–4]. As oviparous animals, chickens rely solely on the nutrition provided by the egg post-separation from the mother, rendering maternal effects critical from the embryonic to early developmental stages in chicks. However, the patterns of these changes remain largely unexplored[5]. Our previous research identified the chicken liver as a target organ influenced by maternal effects at both the transcriptional and epigenetic levels[6,7]. The liver, being the most crucial metabolic organ in chickens, facilitates fat and protein metabolism, as well as the storage of vitamins and minerals, playing a pivotal role in hematopoiesis and glucose and lipid metabolism during embryonic development[8]. Yet, systematic analyses of the transcriptional diversity in the liver across generations, from breeders to embryos to offspring, are scarce.

During the reprogramming process of avian embryonic development, epigenetic modifications of specific genes can undergo aberrations. Mutations in genes encoding epigenetic regulatory proteins may also occur. These changes represent critical phases where maternal effects are exerted[9]. Epigenetics plays a significant role in regulating complex cellular networks. They are involved in key physiological processes such as chromatin assembly and mRNA splicing. The distribution of epigenetic regulators within both the cytoplasm and nucleus is closely associated with their mechanisms of action[10,11]. Notably, genes are intimately linked to embryogenesis and development, with maternally inherited LncRNAs being abundantly present in early embryos[9]; in chicken embryos, there is a marked increase in the

expression of mRNA during developmental stages, suggesting that maternal effect contributes to maintaining pluripotency in stem cells and plays a role in embryonic development[6]. Furthermore, studies indicate that the duration of maternal effects in birds is relatively short[12], which may be closely related to the efficiency of early chick development. However, the changes in marker genes involved in this process and the underlying mechanisms remain largely unclear.

In the production cycle of broiler breeders, individuals entering the late laying phase exhibit significant declines in egg production and increased body fat deposition, impacting liver lipid metabolism and other metabolic pathways[13]. By contrast, during rapid cell division and differentiation, the liver of hatching chicken embryos showcases an efficient energy metabolism system and biosynthesis activities, laying the foundation for subsequent life stages[14]. Furthermore, at 21 d post-hatch, during a period of rapid growth, the liver's functions in energy and protein/amino acid metabolism, lipid metabolism adjustment, vitamin and mineral storage, and immune regulation are crucial to support this growth rate[15].

To delve into the temporal and spatial variations in the liver transcriptomes of broiler chickens at different developmental stages and identify key transcription factors associated with economic traits, particularly metabolic efficiency, this study analyzed 23 high-throughput RNA-Seq libraries from liver tissue samples of AA strain broiler breeders, offspring at the E19 embryonic stage, and 21-day-old post-hatch offspring. Through this approach, we aim to gain a deeper understanding of the functional changes in the liver across developmental stages and their potential impacts on overall health and productivity, thereby uncovering key gene expression patterns that influence liver health and metabolic characteristics.

Materials and methods

Animals and sample collection

The experiment utilized late-laying 57-week-old broiler breeders and their male broiler offspring, ROSS 308, raised under standardized conditions at the Meat Chicken Science and Technology Backyard (Zhuozhou, China). At the end of 65 weeks, liver samples were collected from eight selected broiler breeders. Fertilized eggs were collected, and incubated, and only male offspring chicks were used in this study. Samples were taken on the 19th day of embryonic development for collection and sex determination (seven were selected), and chicks were raised until the age of 21 d, at which point liver samples from eight selected individuals were collected. The samples were immediately frozen in liquid nitrogen and stored at −80℃ for further analysis. The experimental workflow is illustrated in Fig. 1.

Total RNA isolation

The liver samples were collected and immediately placed in RNase-free centrifuge tubes, followed by rapid freezing in liquid nitrogen. Total RNA

isolation was performed using 100 mg tissue samples and 1 mL Trizol reagent (Vazyme #R701), adhering to the protocols provided by the supplier. To evaluate the RNA integrity and purity, a NanoDrop micro-volume spectrophotometer (Thermo Fisher, Wilmington, USA) was utilized, following the procedure outlined by Gao et al.[16]. This procedure guaranteed the structural integrity and dependability of the RNA specimens before further examination.

RNA-Seq library construction and sequencing

Total RNA that met the criteria, with a RIN value of ≥ 7 as determined by the Agilent 2100 Bioanalyzer, was utilized for mRNA library preparation for sequencing[17]. To precisely quantify the molar concentration of the constructed libraries, qPCR with standards was conducted, employing the KAPA Library Quantification Kit (Cat no. KK4602) as recommended by Illumina, ensuring accurate library loading volumes for sequencing. The libraries were then hybridized to a Flowcell using the TruSeq Rapid PE Cluster Kit on a cBot, followed by clustering on the HiSeq2500 system. This step involves immobilizing the library molecules on Flowcell primers and undergoing bridge PCR amplification before sequencing. Illumina's data collection software managed the sequencing operation.
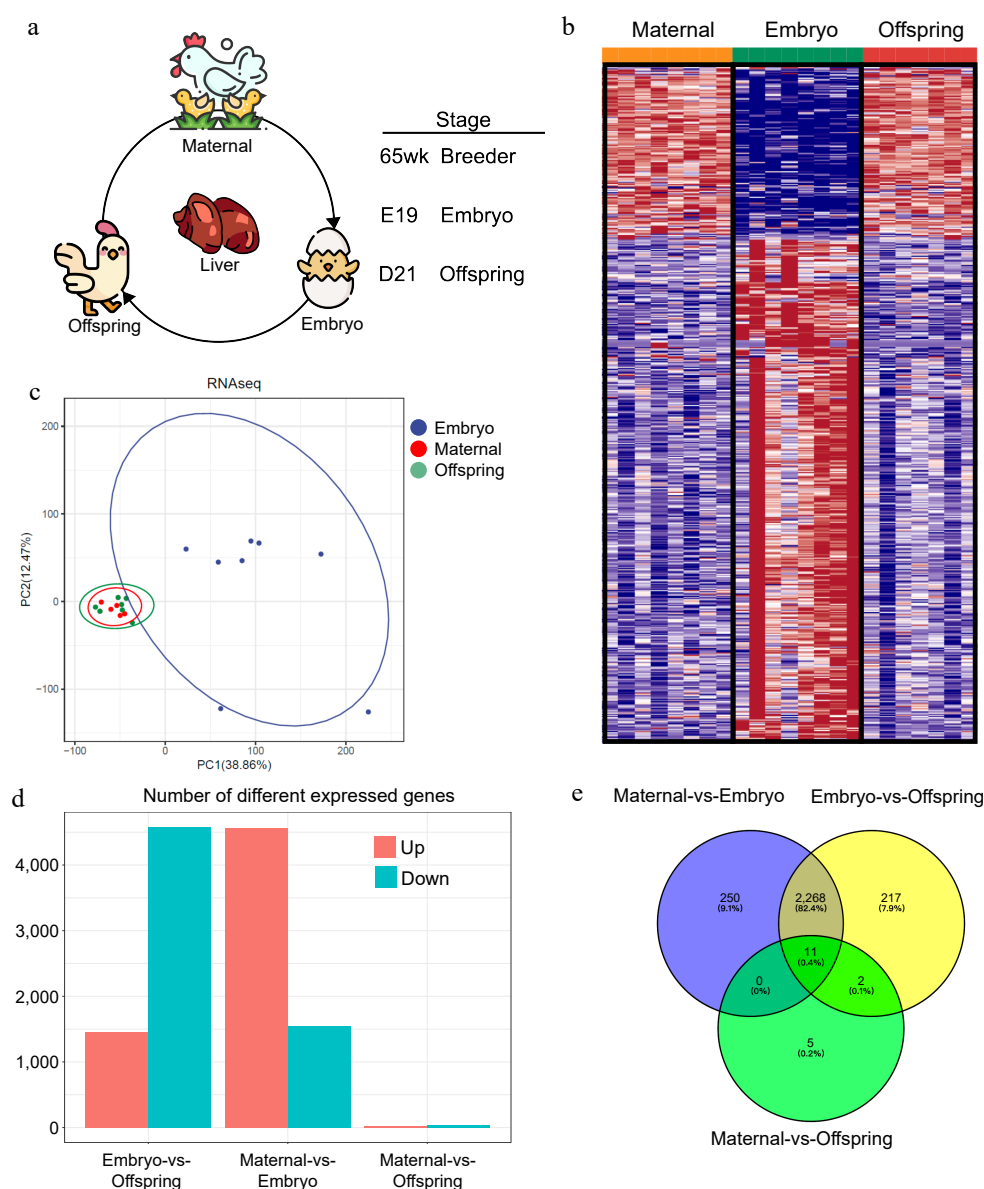
Fig. 1 Liver transcriptome profiles of maternal, embryo, and offspring stages. (a) Workflow diagram, (b) differential gene expression profiles across the three stages, (c) PCoA plot of sample distribution, (d) number of differentially expressed genes between stages, (e) Venn diagram of differentially expressed genes.

Gene expression analysis

Gene expression differentiation analysis employed DESeq2[18], adhering to the approach outlined by Fan et al.[6]. Differentially expressed genes were identified by setting the criteria to an absolute log2 |fold change| ≥1 and a Q-value ≤ 0.05. To assess the expression correlation among samples, the Pearson correlation coefficient for all gene expression data was calculated. For intra-group comparisons, only Pearson correlation coefficients exceeding 0.80 were considered, denoting superior repro-ducibility. Prediction pipeline for lncRNA transcripts: (1) Cufflinks were used to assemble the transcripts for each sample. (2) Cuffmerge was used to merge the transcripts predicted from all individuals. (3) The merged transcripts were compared with the known protein-coding transcripts downloaded from Ensembl using Cuffcompare, filtering out lncRNA loci that encode proteins. (4) Transcripts shorter than 200 bp and with fewer than two exons were removed. (5) CNCI was used to predict the coding potential of transcripts, and transcripts with coding potential were removed. (6) BLAST was used to further filter out transcripts with coding potential by comparing them with the NCBI protein database. Based on the FPKM values of lncRNA expression, differential expression analysis between samples was performed using the DEGseq package. The differential lncRNAs were screened using the statistical methods of fold-change (expression difference multiple) and Fisher's exact test.

Functional enrichment analysis

Enrichment analysis was executed with the entire genome serving as the background set, focusing on the biological process (BP) category of Gene Ontology (GO) for the functional assessment. GO terms were deemed significant if they achieved a P value < 0.01 and were associated with ≥ 2 genes.

Time analysis

Short Time-series Expression Miner (STEM), Using STEM, time-series gene expression data was normalized and analyzed by clustering genes into predefined temporal expression profiles. Parameters such as the number of profiles and FDR correction were set, and the analysis identified significant profiles based on gene expression trends across time points. The results were then visualized, and significant profiles were subjected to Gene Ontology (GO) enrichment analysis to identify related biological processes. Key findings included the identification of gene clusters with distinct temporal expression patterns and their associated GO terms.

Results

Expression of DEGs in the liver across maternal-embryo-offspring stages

To delineate the RNA expression profiles involved in liver function during maternal effects in broiler breeders (Fig. 1a,b), high-throughput RNA sequencing generated 185.88 GB of clean data from 23 liver samples, encompassing both maternal and offspring stages, specifically embryos at day 19 of gestation and chicks 21 d post-hatch. Each sample contributed 7.9 GB of clean data, with a Q30 base percentage consistently exceeding 91.71% (Table 1). A total of 19,031 genes were identified across the liver samples. Clean reads were aligned to the reference chicken genome using HISAT, resulting in a mapping success rate between 90.83% and 93.35% (Table 1). The identification of differentially ex-pressed genes (DEGs) was grounded on comparative analyses and their expression levels across the various samples. A comprehensive array of DEGs was identified in the liver tissue (Table 2, Supplementary Fig. S1a). Principal component analysis (PCA) was employed to model the distri-bution and segregation trends among the maternal, embryonic, and offspring comparison groups, illustrating distinct clustering patterns (Fig. 1c). A Venn diagram further emphasized the shared and unique gene expression alterations occurring across the maternal, embryonic, and offspring stages. When considering these stages as distinct entities, pairwise differential expression analyses revealed that the maternal versus embryonic stages exhibited the highest number of differentially expressed genes, followed by the embryonic versus offspring stages, with the least differential expression observed between the maternal and offspring stages (Fig. 1d, e).

Differential GO analysis of DEGs in the maternal effect process

To further elucidate the characteristic DEGs involved in the liver's role during maternal effects, the top 10 DEGs in the maternal-embryo and embryo-offspring comparisons were identified, including *IGFBP1*, *ABI3BP*, *DIO3*, *PDK4*, *IGFALS*, *IGF1*, among others (Tables 3 & 4). In

Table 1. RNA-seq read statistics.

Sample name	Clean_reads	Raw_bases	Q20	Q30	GC_content	HISAT mapped	HISAT Uniquely mapped
Breeder1	38362812	11040224580	96.53%	93.16%	51.99%	88.5%	75.78%
Breeder2	37847708	10940622840	96.28%	92.59%	56.15%	72.49%	52.58%
Breeder3	34672132	9846336276	96.52%	93.10%	51.41%	89.35%	77.37%
Breeder4	40482362	11592999936	96.48%	93.04%	51.51%	89.92%	76.84%
Breeder5	37196291	10296891108	96.86%	93.68%	49.86%	92.12%	82.17%
Breeder6	40958805	12072458664	96.59%	93.12%	59.16%	59.57%	32.85%
Breeder7	35709829	10510141572	96.47%	92.96%	56.03%	75.07%	55.02%
Breeder8	33669242	9914908752	96.79%	93.69%	51.39%	90.98%	78.82%
Embryo1	40485557	11654523972	95.90%	91.83%	52.50%	89.67%	74.26%
Embryo2	37432078	11069231292	96.48%	93.04%	51.34%	88.79%	74.68%
Embryo3	39065142	11126909556	96.26%	92.59%	51.62%	88.44%	75.25%
Embryo4	35508865	10322469360	96.30%	92.65%	51.73%	90.69%	77.44%
Embryo5	37076246	10837283688	96.46%	92.94%	52.48%	89.48%	74.28%
Embryo6	39932521	11473593264	96.23%	92.53%	51.41%	89.88%	77.24%
Embryo7	43619518	12556149732	96.36%	92.82%	51.46%	90.88%	76.31%
Embryo8	36859302	10562648292	96.14%	92.44%	53.36%	76.34%	58.07%
Offspring1	35823274	10444364028	96.48%	93.12%	50.66%	91.04%	78.7%
Offspring2	38678468	11108159496	96.39%	92.82%	53.96%	81.18%	63.04%
Offspring3	38105455	11117678544	96.33%	92.70%	55.17%	79.53%	62.3%
Offspring4	38509142	11038901832	95.75%	91.71%	51.52%	88.96%	74.73%
Offspring5	38362812	11040224580	96.53%	93.16%	51.99%	88.5%	75.78%
Offspring6	37847708	10940622840	96.28%	92.59%	56.15%	72.49%	52.58%
Offspring7	34672132	9846336276	96.52%	93.10%	51.41%	89.35%	77.37%

Table 2. Pairwise comparison of up and downregulated DEGs between maternal, embryo and offspring chickens in liver.

	Compare group	Down	Up	Total
Liver	Maternal-vs-Embryo	1540	4569	6109
	Embryo-vs-Offspring	1449	4578	6027
	Maternal-vs-Offspring	26	38	64

addition, 2,090 differentially expressed long non-coding RNAs (LncRNAs) were detected, each longer than 1,000 nucleotides (Supplementary Table S1). The distribution of DEGs in the liver are visually depicted in the volcano plots (Fig. 2a).

Enrichment analysis was also performed to pinpoint key genes and pathways implicated in the maternal effect. The liver undergoes a functional transition from cell development and differentiation during the embryonic stage to glucose and lipid metabolism and immune function by the 21-d post-hatch stage. Gene Ontology (GO) enrichment analysis revealed significant biological pathways, including those related to multicellular organism development, synaptic transmission (GABAergic), adherens junction organization, cholesterol biosynthetic process, unsaturated fatty acid biosynthetic process, immune response, and the B cell receptor signaling pathway (Fig. 2b, Fig. 3a & b).

Expression patterns of developmentally related genes across three stages

Based on the analysis of RNA expression profiles across the three stages, 20 characteristic genes involved in the regulation of cell proliferation and

Table 3. Maternal-vs-embryo Top 10 DEGs.

Gene	Log2FC	*p*−value	Regulation
IGFBP1	−3.4921	< 0.0001	down
ABI3BP	−4.4975	< 0.0001	down
MT3	−4.7465	< 0.0001	down
DIO3	−4.8438	< 0.0001	down
CHRNA7	−5.4058	< 0.0001	down
TTLL2	−5.4702	< 0.0001	down
NEGR1	−6.8204	< 0.0001	down
SREBF2	−2.9490	6.11E-303	down
PDK4	−2.8915	3.08E-300	down
IRF1	−3.0829	4.15E-299	down

IGFBP1, Insulin-like Growth Factor Binding Protein 1; *ABI3BP*, ABI Family Member 3 Binding Protein; *MT3*, Metallothionein 3; *DIO3*, Deiodinase Iodothyronine Type III; *CHRNA7*, Cholinergic Receptor Nicotinic Alpha 7 Subunit; *TTLL2*, Tubulin Tyrosine Ligase Like 2; *NEGR1*, Neuronal Growth Regulator 1; *SREBF2*, Sterol Regulatory Element Binding Transcription Factor 2; *PDK4*, Pyruvate Dehydrogenase Kinase 4; *IRF1*, Interferon Regulatory Factor 1.

Table 4. Embryo-vs-Offspring Top 10 DEGs.

Gene	Log2FC	*p*−value	Regulation
MT3	5.1899	< 0.0001	up
ABI3BP	4.5664	< 0.0001	up
IGFBP1	4.1745	< 0.0001	up
HMGCR	−3.1252	< 0.0001	down
IGFALS	−3.7697	< 0.0001	down
IGFBP4	−3.8229	< 0.0001	down
FASN	−5.1979	< 0.0001	down
IGF1	−6.3826	< 0.0001	down
CDKN2B	−6.4335	< 0.0001	down
ELOVL2	−6.2947	< 0.0001	down

MT3, Metallothionein 3; *ABI3BP*, ABI Family Member 3 Binding Protein; *IGFBP1*, Insulin-like growth factor-binding protein 1; *HMGCR*, 3-Hydroxy-3-Methylglutaryl-CoA Reductase; *IGFALS*, Insulin-like growth factor-binding protein; acid labile subunit; *IGFBP4*, Insulin-like growth factor-binding protein 4; *FASN*, Fatty Acid Synthase; *IGF1*, Insulin-like growth factor 1; *CDKN2B*, Cyclin Dependent Kinase Inhibitor 2B; *ELOVL2*,ELOVL Fatty Acid Elongase 2.

organ development were successfully identified (Fig. 4). Key genes such as *IGF1*, *IGFBP1*, *IGFBP2*, *IGFBP4*, *IGFALS*, and *HMGCR* were significantly upregulated in the liver tissues of both embryos and offspring, with expression levels being particularly higher in the embryonic stage. Additionally, critical genes regulating lipid metabolism, including *FASN*, *ELOVL2*, *ELOVL5*, *ELOVL6*, *SREBF2*, *ACACA*, and *CYP7A1* were also found to be significantly upregulated in the liver of both embryos and offspring. A correlation analysis of characteristic target genes (including LncRNAs) across the three stages was conducted to gain deeper insights into the regulatory patterns of maternal effects (Fig. 5, Supplementary Table S2).

Time analysis of liver function across three developmental stages

Time-series analysis was utilized to explore the DEGs across the maternal-embryo-offspring stages, aiming to delineate the dynamic changes in liver mRNA expression profiles influenced by maternal effects (Fig. 6a). By comparing DEGs with a Q value ≤ 0.05 and an absolute log2|fold change| ≥ 1 across the various stages, the DEGs were organized into six distinct clusters, with expression patterns predominantly following 'high-low-high' and 'low-high-low' trends (Fig. 6b).

In Cluster 1, KEGG pathway enrichment analysis revealed a significant association with the 'Toll-like receptor signaling pathway', where key characteristic genes included *TLR2*, *TLR4*, *TLR5*, *MyD88*, and *NF-κB*. The 'Lysosome' pathway was also enriched, with *CTSD* (Cathepsin D) and *LAMP1* (Lysosomal Associated Membrane Protein 1) identified as pivotal genes, alongside the 'C-type lectin receptor signaling pathway'. Furthermore, the Gene Ontology (GO) categories in this cluster were significantly enriched for activities such as GTPase activator activity, actin filament binding, small GTPase binding, and protein tyrosine kinase activity (Fig. 7a−c).

In Cluster 2, KEGG analysis highlighted enrichment in the 'Neuroactive ligand-receptor interaction' pathway, with *GABRA* identified as a characteristic gene, as well as in the 'Calcium signaling pathway' and 'ECM-receptor interaction'. GO categories in this cluster were enriched in functions such as 'Calcium ion binding', 'nucleotide binding', 'synapse', 'neuron projection', 'glutamatergic synapse', and 'microtubule cytoskeleton', with *TUBB*, *MAPs*, and *KIFs* identified as key genes (Supplementary Table S3). The remaining four clusters also displayed enrichment in GO categories related to metabolism, development, and immune function, reflecting dynamic processes that occur throughout the maternal-embryo-offspring transition (Fig. 8a−c, Supplementary Fig. S1b).

Discussion

Chicken embryos require 21 d to develop and hatch, constituting one-third of the overall grow-out period. Consequently, early developmental stages are crucial in determining the final body weight at market. The maternal effects exerted by breeder hens play a decisive role during embryonic development[19]. The development of the liver during the embryonic stage is critical for broiler growth and is regulated by factors such as maternal nutrition and maternal epigenetic modifications[7]. Throughout embryonic development, the liver's metabolic functions transition from an early reliance on anaerobic glycolysis to a dependence on aerobic respiration. As development progresses, the liver's capacity for glucose metabolism strengthens, with gluconeogenesis and glycogen metabolism becoming increasingly refined[20]. Simultaneously, lipid metabolism becomes more active, with enhanced fatty acid oxidation capabilities[21]. However, the core regulatory genes involved in liver development, as well as the mechanisms underlying their changes during maternal effects, remain inadequately understood. To address this gap, a comprehensive transcriptome analysis was performed to elucidate the gene expression changes and related functional pathways that charac-
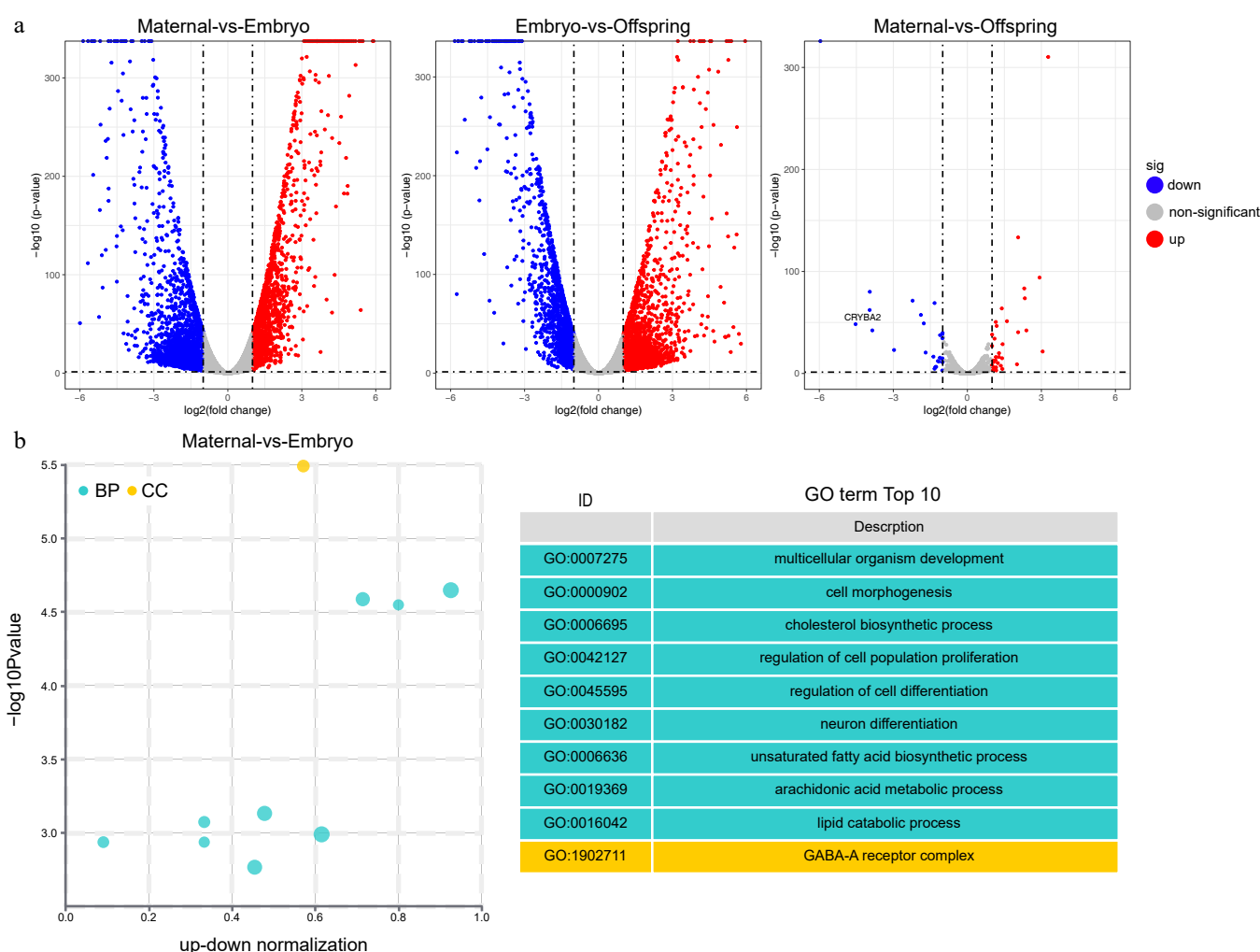
Fig. 2 GO enrichment analysis of differentially expressed genes (DEGs). (a) Volcano plot, (b) top 10 GO pathways enriched in DEGs between maternal and embryo stages. The cyan color represents GO's Biological Process (BP) pathways, while the yellow color represents GO's Cellular Component (CC) pathways.

terize liver development from breeder chickens, through embryos, to offspring broilers. This analysis offers critical insights into the molecular underpinnings of liver development, laying a solid foundation for future research into the maternal effects influencing growth and development in broiler chickens.

Based on the comparative analysis of liver expression profiles between breeder hens and E19 chicken embryos, the top 10 significantly differentially expressed genes were identified, including *IGFBP1*, *ABI3BP*, *MT3*, *DIO3*, *CHRNA7*, *TTLL2*, *NEGR1*, *SREBF2*, *PDK4*, and *IRF1*. Previous studies have demonstrated that *IGFBP1* plays a crucial role in regulating cell growth, differentiation, and metabolism by modulating the activity of insulin-like growth factors (IGFs)[22]. Additionally, *DIO3* is involved in the regulation of thyroid hormones and promotes embryonic development by inactivating T3 and T4[23]. Through a comparative analysis of liver expression profiles between E19 chicken embryos and D21 broiler chickens, *MT3*, *ABI3BP*, and *IGFBP1* were identified as characteristic genes highly expressed in E19 embryos. *ABI3BP* is involved in cytoskeletal reorganization and extracellular matrix signaling, playing a crucial role in influencing cell migration and tissue morphology during embryonic development[24]. Furthermore, a significant upregulation of key genes regulating lipid metabolism, *FASN*, and *ELOVL2*, was observed in D21 broilers. *FASN* acts as a pivotal enzyme in fatty acid synthesis, is closely associated with adipogenesis, and is highly expressed in metabolically active liver tissues[25]. *ELOVL2* is essential for the elon-

gation of fatty acids and is integral to the synthesis of polyunsaturated fatty acids[26]. The elevated expression of these genes is intricately linked to the nutritional demands of different developmental stages; E19 chicken embryos primarily rely on glycogen metabolism for energy, whereas D21 broilers, during their rapid growth phase, necessitate extensive lipid metabolism to satisfy their energy requirements[27]. Maternal nutrition plays an important role in shaping the offspring's metabolic system. For instance, lipid and carbohydrate metabolism are closely related to the mother's fat and carbohydrate intake. Nutritional imbalances could lead to metabolic disorders or abnormal fat deposition in the offspring.

In the GO analysis of DEGs between maternal and embryo samples, significant enrichment in terms related to multicellular organism development, cell morphogenesis, and regulation of cell differentiation were observed. These biological processes are critical for ensuring the correct spatial arrangement of cells within tissues and organs during embryonic development, allowing cells to acquire the necessary functional characteristics[28]. Epigenetic regulation plays a crucial role in influencing both embryonic and offspring development[29]. In this study, 2,090 differential LncRNAs were identified and an association analysis performed with differential mRNAs, uncovering relationships of synergistic regulation and mutual inhibition. Previous studies have suggested that the maternal effects in breeder hens can influence the transcriptional levels of key genes such as *MyoD* in chicken embryos through LncRNA-mediated cis-regulation, thereby impacting embryonic metabolic development and immune processes[30].

a

b
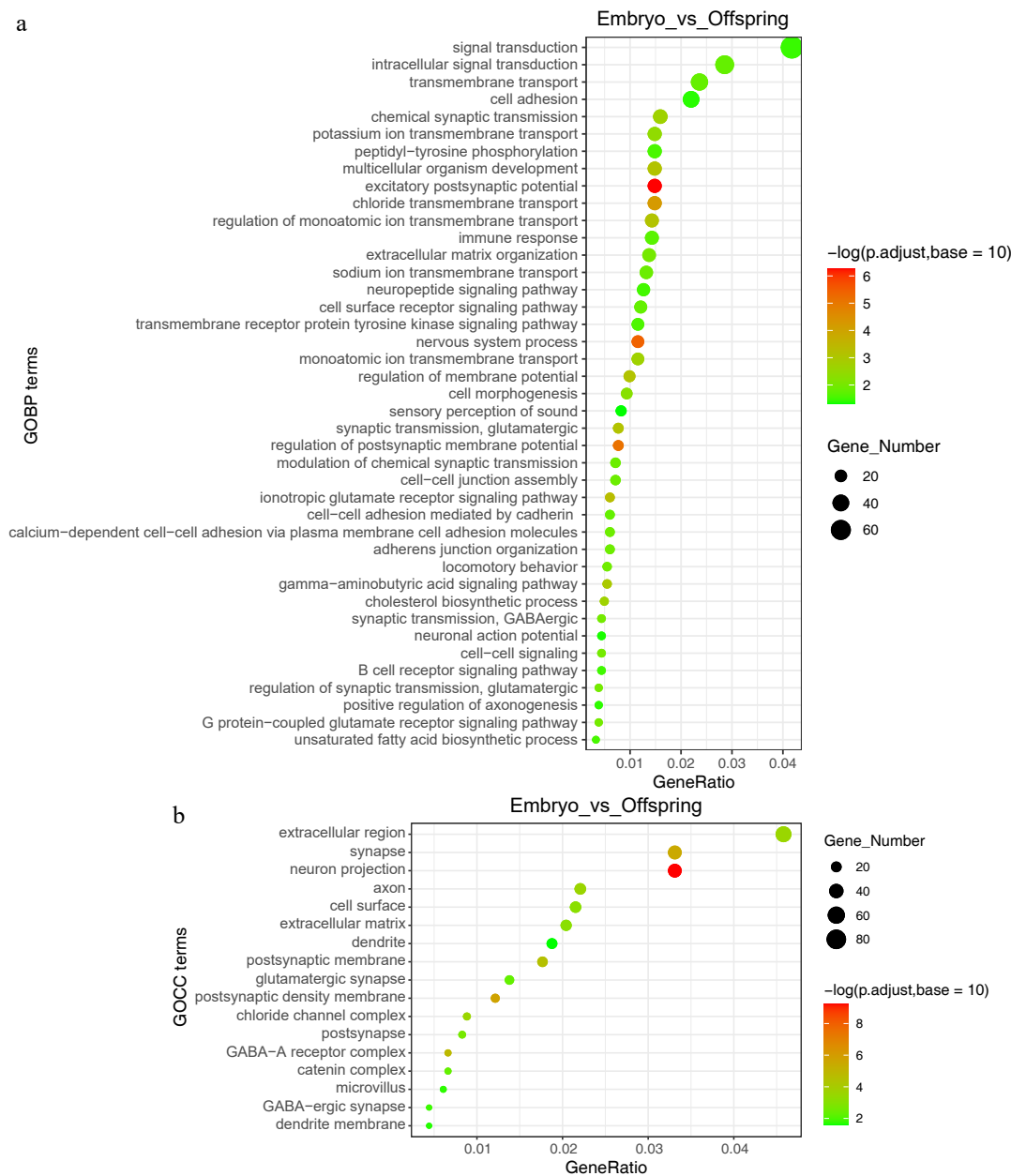
Fig. 3 GO functional annotation analysis of embryo vs offspring stages. (a) Enriched functions at the GOBP (Gene Ontology Biological Process) level, (b) enriched functions at the GOCC (Gene Ontology Cellular Component) level.
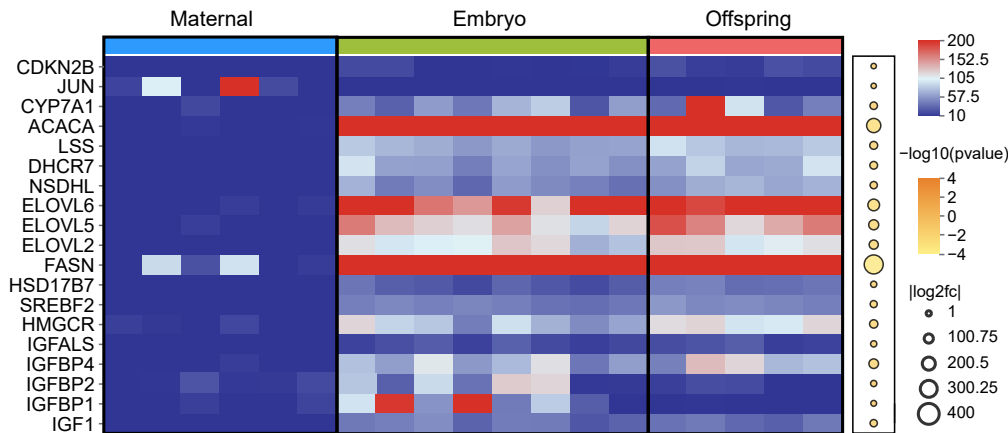
Fig. 4 Expression of DEGs in maternal, embryo, and offspring liver tissues. The red and blue squares represent FPKM values, with darker red indicating higher expression levels. Upregulated DEGs are marked in dark yellow, while downregulated DEGs are shown in white.
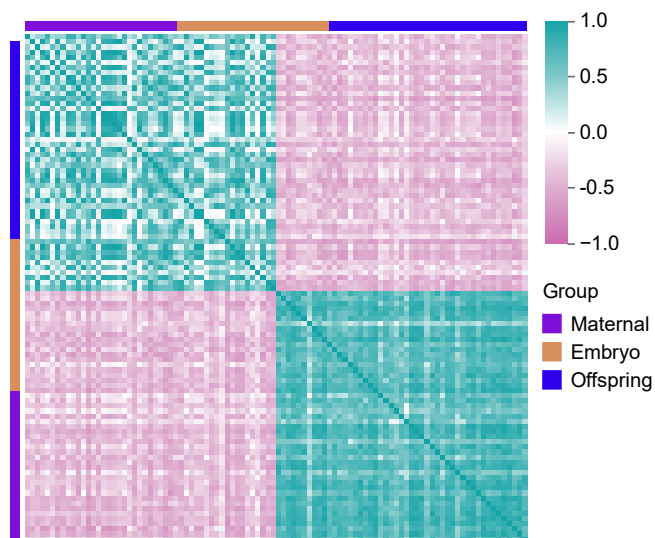
Fig. 5 Correlation analysis of DEGs in maternal, embryo, and offspring stages. Pink represents negative correlations, while cyan indicates positive correlations.

Moreover, the present study analyzed DEGs across maternal, embryo, and offspring stages, and constructed an inter-regulatory GO network based on the distinct metabolic characteristics of each stage. This network provides a theoretical basis for further research into the mechanisms by which maternal effects regulate offspring development. The distinct metabolic characteristics observed across maternal, embryo, and offspring stages suggest that maternal effects play a crucial role in shaping the developmental trajectory and functional maturation of offspring.

To further investigate the maternal effects on liver expression profiles, the top 20 DEGs were identified by selecting those with low expression levels in breeder hens and high expression levels in embryos and offspring broilers. Among these, the IGF family members, including *IGF1*, *IGFBP1*, *IGFBP2*, *IGFBP4*, and *IGFALS*, were found to exhibit consistently high expression from the E19 embryo stage to D21 broilers. The expression of *IGF1* is subject to regulation by DNA methylation and non-coding RNAs, where maternal malnutrition can lead to decreased levels of fetal *IGF1* through epigenetic modifications, subsequently inhibiting fetal growth and development[31]. *IGFBP1*, *IGFBP2*, and *IGFBP4* modulate the activity of growth factors by binding to *IGF1* and *IGF2*[32,33]. Additionally, maternal metabolic conditions, such as obesity or diabetes, can suppress the activity of these IGFBPs through mechanisms involving miRNA and DNA methylation, thereby reducing the secretion of growth factors and potentially affecting the growth and development of the offspring[34,35]. Additionally, lipid metabolism-related genes, such as *FASN*, *ELOVL2*, *ELOVL5*, *ELOVL6*, and *ACACA*, were found to be consistently highly expressed from the E19 embryo stage to D21 broilers. Research has shown that *FASN* is subject to indirect regulation by LncRNA H19, which promotes the process of fatty acid synthesis[36]. Although there are few reports on the epigenetic regulation of the *ELOVL* gene family, these genes play a crucial role in the elongation of fatty acids. Their activity is modulated under varying lipid nutritional conditions, thereby enhancing the elongation process to support lipid metabolism[37]. These findings further highlight the critical role of maternal effects in regulating the development of the offspring's liver.

To elucidate the gene regulatory mechanisms and temporal characteristics during maternal effects, a time-series analysis of DEGs was constructed across the maternal, embryo, and offspring stages. The enriched DEGs predominantly followed 'high-low-high' and 'low-
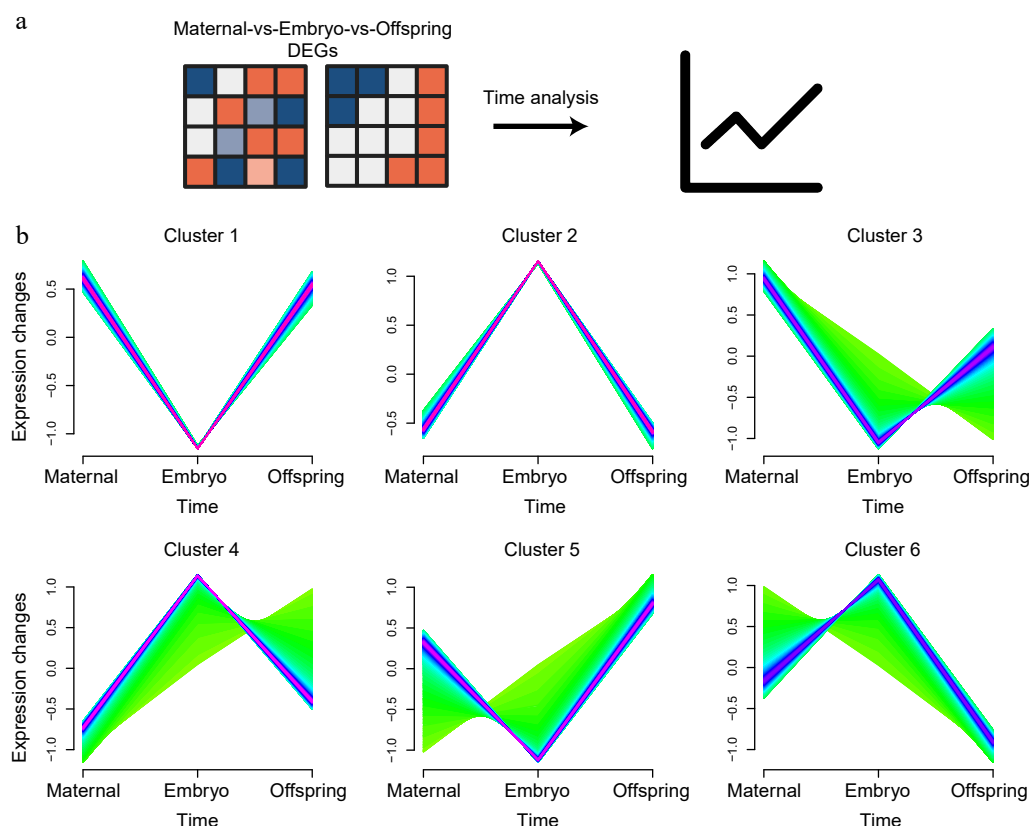
Fig. 6 Time analysis of liver expression profiles across maternal, embryo, and offspring stages. (a) Workflow diagram of the time analysis. (b) Cluster analysis of DEGs across the three stages, showing six distinct clusters.
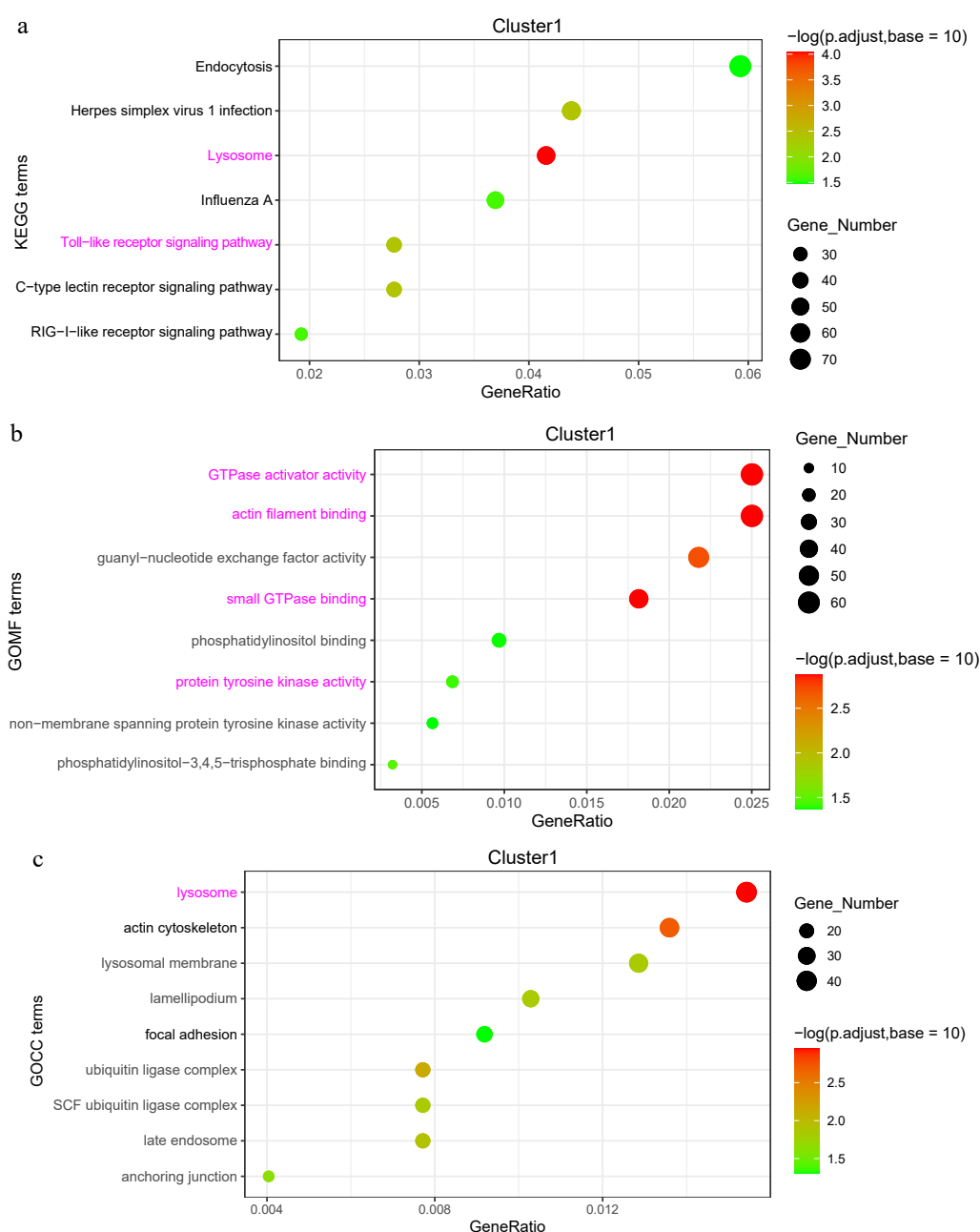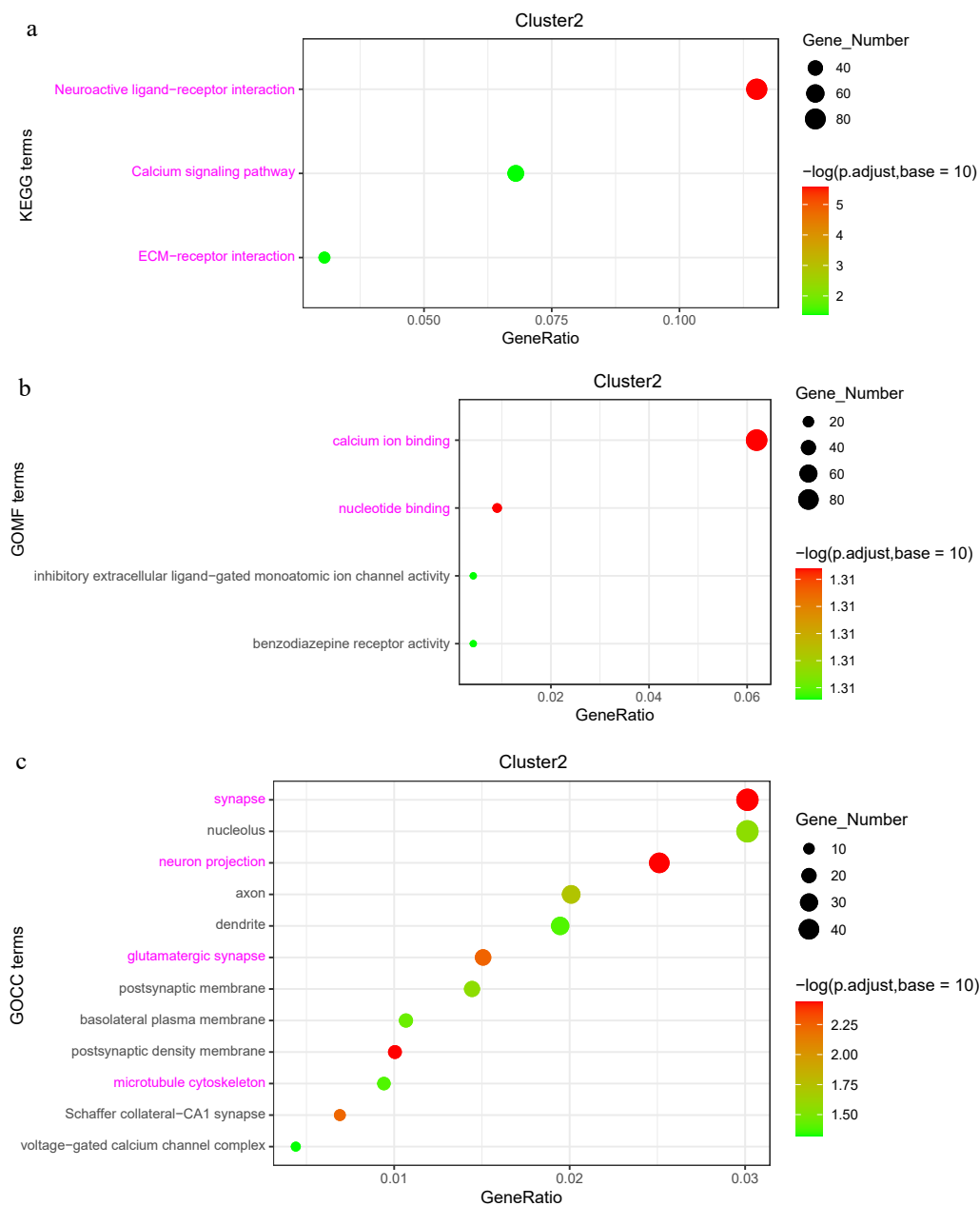
Fig. 7 Analysis of DEGs in the 'high-low-high' expression pattern (Cluster 1). (a) KEGG enrichment analysis of DEGs. (b) GOMF (Gene Ontology Molecular Function) enrichment analysis of DEGs. (c) GOCC (Gene Ontology Cellular Component) enrichment analysis of DEGs.

high-low' expression patterns. In the 'high-low-high' pattern, the enriched genes were primarily associated with immune function. The 'Lysosome pathway' was particularly significant in both the maternal and D21 offspring broiler stages, where cellular renewal, tissue repair, and immune responses depend heavily on lysosomal activity[38]. During the chicken embryo development stage, the primary cellular functions are rapid proliferation and differentiation, which leads to a relatively lower demand for lysosomal degradation. In this context, key genes such as Lysosomal Associated Membrane Protein 1 (*LAMP1*) and Cathepsin D (*CTSD*) were identified as crucial players. *LAMP1* is essential for lysosomal transport, facilitating the movement of enzymes and proteins within lysosomes. *CTSD*, on the other hand, plays a significant role in lysosomal protein degradation, breaking down cellular waste and damaged proteins. These genes are vital for maintaining lysosomal function, which is critical for cellular homeostasis and metabolic regulation[39,40]. These findings provide deeper insights into

the gene regulatory dynamics influenced by maternal effects throughout different developmental stages. The 'Toll-like receptor signaling pathway' plays a crucial role in recognizing pathogen-associated molecular patterns (PAMPs) and initiating immune responses[41]. During the maternal and offspring stages, exposure to external environments increases the likelihood of encountering bacteria and viruses, which in turn activates the expression of relevant characteristic genes involved in this pathway[42]. However, during the chicken embryo developmental stage, the immune system remains immature, and the relatively enclosed embryonic environment limits exposure to pathogens, resulting in fewer opportunities for activation of this pathway. The present study revealed that *MyD88*, TLR family genes, and *NF-kB* were significantly downregulated in chicken embryos, indicating that the immune response in chicken embryos relies more heavily on maternally provided antibodies and other protective mechanisms, resulting in a lower requirement for TLR pathway activation.

Fig. 8 Analysis of DEGs in the 'low-high-low' expression pattern (Cluster 2). (a) KEGG enrichment analysis of DEGs. (b) GOMF (Gene Ontology Molecular Function) enrichment analysis of DEGs. (c) GOCC (Gene Ontology Cellular Component) enrichment analysis of DEGs.

In the 'low-high-low' expression pattern, the enriched genes were predominantly linked to cell proliferation and organogenesis. The 'Neuroactive ligand-receptor interaction pathway', which reflects the functional demands of the nervous system at various developmental stages showed that during the chicken embryo stage, neurotransmitter activity is primarily concentrated on early neural network formation and initial signal transduction[43]. The differentially upregulated gene Gamma-Aminobutyric Acid Receptor Subunit Alpha (*GABRA*), a major receptor for GABA plays a critical role in the synthesis of neurons in chicken embryos[44]. The 'Calcium signaling pathway' plays a crucial role during chicken embryo development, particularly in regulating rapid cell division, differentiation, and the formation of the muscle system[45]. As breeder hens and offspring broilers reach maturity, the rate of cell division and differentiation significantly decreases, leading to reduced dependence on calcium signaling[46]. Concurrently, metabolic processes undergo reprogramming, especially in offspring broilers, where the focus shifts toward energy metabolism, muscle

hypertrophy, and enhanced production performance[27].

Significant enrichment of the 'Microtubule cytoskeleton' was also observed during the chicken embryo stage, where it plays a pivotal role in maintaining cell structure and supporting cell proliferation[47]. During this developmental phase, cells require frequent structural reorganization and dynamic changes, and the high expression of microtubule cytoskeleton components ensures these processes occur efficiently, thereby facilitating organ formation and functional development[48]. Among the enriched genes, *TUBB* (Beta-tubulin) stands out as a core gene involved in maintaining cell morphology, driving cell division, mediating endocytosis, and supporting intracellular transport[49]. Additionally, *MAPs* (Microtubule-associated proteins) are crucial for regulating microtubule growth by binding to microtubules, ensuring the proper functioning of the cytoskeleton and thereby maintaining cellular integrity and facilitating development[50]. In summary, the distinct gene regulatory networks observed in this study reflect the dynamic changes in gene expression and function

across different developmental stages. Immune-related pathways become increasingly active as broilers grow, supporting the complex functions of a mature physiological system. In contrast, the pathways enriched in chicken embryos are primarily focused on fundamental developmental functions, such as cell division, migration, and nervous system development. These differences in gene expression underscore the varying metabolic and physiological processes occurring at each stage of development. Moreover, these findings provide a foundational basis for further investigation into the epigenetic regulation of maternal effects in breeder hens, offering insights into how these effects influence the development and function of offspring.

Conclusions

This study, by comparing liver gene expression profiles among breeder hens, chicken embryos, and offspring broilers, has highlighted the pivotal role of maternal effects in embryonic development. Significantly differentially expressed genes such as *IGFBP1*, *ABI3BP*, and *DIO3*, were identified which are crucial for glucose metabolism, lipid metabolism, and cytoskeletal reorganization. Time analysis revealed two primary gene expression patterns: 'high-low-high' and 'low-high-low'. The former pattern, associated with immune function, includes genes like *LAMP1* and *CTSD* that are highly expressed in breeder hens and offspring broilers, indicating enhanced immune activity. The latter pattern includes genes such as *GABRA* and *TUBB*, which are highly expressed in chicken embryos, supporting neural network formation and cytoskeletal reorganization. In summary, maternal effects influence the expression of key genes in embryonic development through epigenetic regulation. Understanding this mechanism is crucial for improving broiler production performance. Future research should focus on exploring the relationship between maternal nutrition and epigenetics to optimize feeding strategies for breeder hens.

Ethical statement

All procedures were reviewed and preapproved by the China Agricultural University Animal Care and Use Committee (Beijing, China), identification number: A070805202-1-1, approval date: 07/08/2023. The research followed the 'Replacement, Reduction, and Refinement' principles to minimize harm to animals. This article provides details on the housing conditions, care, and pain management for the animals, ensuring that the impact on the animals was minimized during the experiment.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Lv Z, Guo Y; data collection: Fan H, Wang H, Chen S, Nie W; analysis and interpretation of results: Gao M, Chen Y; draft manuscript preparation: Gao M. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The data that support the findings of this study are available in the Genome Sequence Archive at the National Genomics Data Center, China National Center for Bioinformation (GSA: CRA016504).

Acknowledgments

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/animadv-0024-0003)

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