ChinaRxiv 中X

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-201711.00722

Effects of Astragalus Polysaccharide on miR-16 Expression in Different Tissues of Breeding Roosters and Functional Prediction Analysis Postprint

Authors: Ren Xiaochun, Li Yulong, Wu Shengru, Lei Xinyu, Guo Wei, Dang Yanna, Gong Yuesheng, Yang Xiaojun

Date: 2017-10-10T00:00:00+00:00

Abstract

This study investigated the effects of Astragalus polysaccharide (APS) on miR-16 expression in different tissues of breeder roosters, combined with GO and KEGG functional enrichment analysis of miR-16 predicted target genes, aiming to explore the potential regulatory effects of APS on different tissue functions. Sixty-four 1-day-old Cobb 500 parent-generation broiler breeder roosters were selected and randomly divided into a control group and an APS group, with 4 replicates per group and 8 chickens per replicate. The control group was fed a corn-soybean meal basal diet, while the APS group was fed the basal diet supplemented with 10 g/kg APS, for a trial period of 40 weeks. The results showed: 1) Compared with the control group, the APS group could increase semen collection volume and effective sperm count, and decrease sperm abnormality rate in breeder roosters, but the differences were not significant ($P>0.05$). 2) miR-16 exhibited tissue-specific expression patterns; APS could significantly or extremely significantly upregulate miR-16 expression in liver, spleen, duodenal mucosa, and ileal mucosa ($P<0.05$ or $P<0.01$), and significantly downregulate miR-16 expression in testis ($P<0.05$). 3) GO enrichment analysis of miR-16 predicted target genes indicated that the predicted target genes were significantly or extremely significantly enriched in biological processes related to material metabolism such as transmembrane transport, ubiquitin-dependent protein degradation, intracellular protein transport, and glycolysis ($P<0.05$ or $P<0.01$). KEGG enrichment analysis showed that miR-16 target genes were significantly enriched in pathways related to organismal material metabolism and cell proliferation and differentiation, such as focal adhesion, insulin signaling pathway, and glycolysis/gluconeogenesis ($P<0.05$), and were also enriched in immune-related pathways including Toll-like receptor signaling pathway and

natural killer cell-mediated cytotoxicity. In conclusion, APS upregulated miR-16 expression in spleen, liver, duodenal mucosa, and ileal mucosa tissues of breeder roosters, and downregulated miR-16 expression in testis; miR-16 predicted target genes were closely associated with material metabolism and immune regulation. APS affects organismal material metabolism and immune regulatory functions by regulating miR-16 expression in different tissues of breeder roosters.

Full Text

Effects and Bioinformatic Analysis of Astragalus Polysaccharide on miR-16 Expression in Different Tissues of Breeder Cocks

REN Xiaochun, LI Yulong, WU Shengru, LEI Xinyu, GUO Wei, DANG Yanna, GONG Yuesheng*, YANG Xiaojun*
(College of Animal Science and Technology, Northwest A&F University, Yangling 712100)

Abstract: This experiment investigated the effects of Astragalus polysaccharide (APS) on miR-16 expression in different tissues of breeder cocks, combined with functional enrichment analysis of GO and KEGG pathways for predicted miR-16 target genes, to explore the potential regulatory roles of APS in different tissues via miR-16. Sixty-four one-day-old Cobb 500 breeder cocks were randomly divided into a control group and an APS group, with 4 replicates per group and 8 cocks per replicate. The control group received a corn-soybean meal basal diet, while the APS group received the basal diet supplemented with 10 g/kg APS for a 40-week experimental period. The results showed: 1) Compared with the control group, APS supplementation improved semen volume and effective sperm number while decreasing sperm abnormality rate, though these differences were not significant ($P>0.05$). 2) miR-16 exhibited tissue-specific differential expression; APS significantly or extremely significantly upregulated miR-16 expression in liver, spleen, duodenum mucosa, and ileum mucosa ($P<0.05$ or $P<0.01$), and significantly downregulated miR-16 expression in testis ($P<0.05$). 3) GO enrichment analysis of predicted miR-16 target genes revealed significant or extremely significant enrichment in biological processes related to material metabolism, including transmembrane transport, ubiquitin-dependent protein degradation, intracellular protein transport, and glycolysis ($P<0.05$ or $P<0.01$). KEGG pathway analysis indicated that miR-16 target genes were significantly enriched in pathways associated with cell proliferation, differentiation, and metabolism, such as focal adhesion, insulin signaling pathway, and glycolysis/gluconeogenesis ($P<0.05$), with additional enrichment in immune-related pathways including Toll-like receptor signaling pathway and natural killer cell-mediated cytotoxicity. In conclusion, APS upregulated miR-16 expression in spleen, liver, duodenum mucosa, and ileum mucosa while downregulating it in testis of breeder cocks. The predicted miR-16 target genes are

closely associated with material metabolism and immune regulation, suggesting that APS affects metabolic and immune regulatory functions by modulating miR-16 expression across different tissues.

Keywords: Astragalus polysaccharide; breeder cocks; miR-16; semen quality; tissue expression; bioinformatics analysis

Corresponding authors: YANG Xiaojun, professor, E-mail: yangxj@nwsuaf.edu.cn; GONG Yuesheng, professor, E-mail: gongyuesheng@sohu.com

---

Astragalus polysaccharide (APS) is a water-soluble heteropolysaccharide extracted from Astragalus membranaceus roots that exhibits multiple biological activities, including regulation of material metabolism, anti-inflammatory effects, antioxidant properties, anti-tumor activity, and immune modulation. As a non-nutritive feed additive, APS effectively improves animal health and production performance in livestock and poultry. Elucidating the underlying mechanisms through which APS regulates animal metabolism and immune function will facilitate more effective application of APS in production practice. APS exerts its regulatory effects by influencing the expression of immune and metabolism-related genes, and the expression of most genes is regulated by miRNAs through RNA-RNA interactions at the post-transcriptional level. Evidence indicates that nutritional components and levels can affect miRNA expression in animals, thereby regulating health status. miRNA expression exhibits tissue specificity and changes with developmental status, playing crucial roles in cell proliferation, differentiation, apoptosis, organism development, metabolism, and immune responses to ensure normal development. Additionally, miRNAs regulate gene expression to adapt to environmental changes, maintaining healthy physiological states. Therefore, under the same nutritional stimulus, the same miRNA may show differential expression patterns, thereby regulating distinct tissue functions.

miR-16 is derived from two precursors, mir-16-1 and mir-16-2, located on chicken chromosomes 1 and 9, respectively. Mature miR-16 is formed through splicing and has been shown to inhibit tumors, participate in immune responses, regulate key enzymes, and affect glucose metabolism. Our previous high-throughput sequencing studies demonstrated that APS significantly reduced miR-16 expression in testicular tissue, suggesting a correspondence between the biological regulatory functions of miR-16 and APS, which led us to select miR-16 as the focus of this study. While most functional studies of miR-16 have been conducted in humans and mice, no reports exist on tissue expression patterns and functions of chicken miR-16 under nutritional regulation. This study examined miR-16 expression across nine tissues in breeder cocks fed APS-supplemented diets, combined with bioinformatic analysis of predicted target genes, to provide insights for future investigations into APS-mediated regulation of metabolism and immune performance through miR-16.

1.1 Experimental Materials

APS: Powder form, 70% purity, purchased from North China Pharmaceutical Co., Ltd.
Experimental animals: One-day-old Cobb 500 breeder cocks, purchased from Beijing Poultry Breeding Co., Ltd.

1.2 Experimental Design

Sixty-four one-day-old Cobb 500 breeder cocks were randomly divided into a control group and an APS group, with 4 replicates per treatment and 8 cocks per replicate. The control group received a corn-soybean meal basal diet, while the APS group received the basal diet supplemented with 10 g/kg APS. All birds received standard immunization, had free access to water, and were fed according to standard protocols. The experimental period lasted 40 weeks.

1.3 Semen Quality Assessment

Semen was collected using the abdominal massage method. Semen collection training was conducted during weeks 38-39 to establish conditioned reflexes. After collection, semen samples were briefly stored in a 37°C water bath before immediate assessment using conventional semen quality evaluation methods. Semen volume (mL), sperm motility (percentage of forward-progressive sperm), sperm density (10 spz/mL), and sperm abnormality rate (%) were measured. Effective sperm number (10 spz) was calculated as the product of semen volume, sperm density, and sperm motility.

1.4 Sample Collection and Total RNA Extraction

Sample collection: At 40 weeks of age, one cock per replicate was randomly selected for sampling. After semen collection via abdominal massage, birds were euthanized by cervical dislocation and slaughtered. Liver, spleen, thymus, testis, and pectoral muscle samples were collected in cryovials. Duodenum, jejunum, and ileum were isolated, rinsed with physiological saline to remove contents, and mucosa was gently scraped with a glass slide into cryovials. All samples were immediately frozen in liquid nitrogen and subsequently stored at -80°C.

Total RNA extraction: Total RNA from semen was extracted using the hot acid phenol method, while total RNA from other tissues was extracted using the Trizol (Takara) method.

1.5 Real-time Quantitative PCR

Bulge-Loop™ miRNA qRT-PCR Primer Set and U6 snRNA qPCR Primer Set (RiboBio, Guangzhou) were used for quantification of miR-16 and U6 snRNA. Reverse transcription of miR-16 and U6 snRNA was performed using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara).

Reverse transcription system (20 L): 5× gDNA Eraser Buffer 2 L, gDNA Eraser 1 L, Total RNA (<1 g), RNase Free dH O to 10 L; incubated at 42°C for 2 min. Then added: 5× PrimeScript Buffer 4 L, PrimeScript RT Enzyme Mix I 1 L, RT primer 2 L, and the previous 10 L reaction mixture, with RNase Free dH O to 20 L. Incubated at 37°C for 15 min, 85°C for 15 s, then cooled to 4°C.

qPCR detection: SYBR® Premix Ex Taq™ II (Takara) was used to detect miR-16 and U6 snRNA expression. Reaction system (20 L): SYBR Premix Ex Taq II (2×) 9 L, Bulge-Loop™ miRNA Forward Primer 0.8 L, Bulge-Loop™ miRNA Reverse Primer 0.8 L, reverse transcription product 2 L, RNase Free dH O 7.4 L. Cycling conditions: 95°C for 30 s; 40 cycles of 95°C for 5 s, 60°C for 30 s, 72°C for 30 s; fluorescence acquisition at 72°C. Three technical replicates were performed for each tissue sample, with U6 snRNA as the internal reference gene.

1.6 miRNA Target Gene Prediction and Enrichment Analysis

TargetScan and miRanda software were used to predict potential target genes of chicken miR-16. The intersection of target genes identified by both algorithms was selected for subsequent bioinformatic analysis. DAVID database (https://david.ncifcrf.gov/) was used for GO enrichment analysis and KEGG pathway analysis of predicted target genes.

1.7 Statistical Analysis

Relative miR-16 expression levels in each tissue were calculated using the 2^(-ΔΔCt) method. Data were analyzed using SPSS 20.0 software with Student' s t-test. Results are expressed as mean ± standard error. P<0.05 was considered statistically significant, and P<0.01 was considered extremely significant.

2.1 Effects of APS on Semen Quality of Breeder Cocks

As shown in Table 1 , compared with the control group, the APS group showed higher semen volume and effective sperm number, and lower sperm abnormality rate, though these differences were not significant (P>0.05). No significant differences were observed in sperm density or sperm motility between the two groups (P>0.05).

Table 1 Effects of APS on sperm quality of breeder cocks

Items	Control group	APS group	P-value
Semen volume (mL)	0.49±0.09	0.58±0.10	>0.05
Sperm density (10 spz/mL)	31.85±4.12	31.78±0.95	>0.05
Sperm motility (%)	95.00±2.89	87.50±2.50	>0.05
Percentage of abnormal sperm (%)	7.00±1.47	5.25±1.25	>0.05
Number of effective sperm (10)	14.11±3.99	15.14±2.45	>0.05

Items	Control group	APS group	P-value

In the same row, values with no letter or the same letter superscripts mean no significant difference (P>0.05).

2.2 Verification of miR-16 Primer Specificity

Based on semen quality assessment, our research group performed miRNA high-throughput sequencing on breeder cock testis and found that APS significantly reduced miR-16 expression in testicular tissue, indicating a correspondence between the biological regulatory functions of miR-16 and APS. Therefore, this study selected miR-16 as the target for subsequent investigation. This experiment used customized stem-loop primers with SYBR dye method to detect miR-16 expression in different tissues of breeder cocks. The melting curves of miR-16 and U6 snRNA and gel electrophoresis detection of some PCR products are shown in Figure 1 [Figure 1: see original paper]. The melting curves of both miR-16 and U6 snRNA showed single peaks, and electrophoresis bands were uniformly distributed around 100 bp, indicating high primer specificity. These results confirm the reliability of subsequent quantitative analyses.

Figure 1 The melting curve of real-time quantitative PCR (A and B) and gel electrophoresis detection of product (C). (A) Melt curve of miR-16; (B) Melt curve of U6 snRNA (internal standard); (C) 1: jejunum; 2: ileum; 3: duodenum; 4: semen; 5: testis; 6: spleen; 7: liver; 8: muscle; 9: thymus; M: DL500 Marker.

2.3 Effects of APS on Relative miR-16 Expression Levels in Different Tissues of Breeder Cocks

As shown in Figure 2 [Figure 2: see original paper], miR-16 was expressed at varying levels across all nine examined tissues. Figure 3 [Figure 3: see original paper] demonstrates that APS significantly or extremely significantly increased miR-16 relative expression levels in spleen, liver, duodenum mucosa, and ileum mucosa ($P<0.05$ or $P<0.01$). In testicular tissue, miR-16 relative expression was significantly decreased in the APS group ($P<0.05$). APS had no significant effects on miR-16 expression in thymus, muscle, semen, or jejunum mucosa ($P>0.05$).

Figure 2 The relative expression level of miR-16 in different tissues of male chicken breeders.

Figure 3 Effects of APS on the relative expression level of miR-16 in different tissues of male chicken breeders. Value columns with no letter superscripts indicate no significant difference ($P>0.05$), different lowercase letters indicate significant difference ($P<0.05$), and different capital letters indicate extremely significant difference ($P<0.01$).

2.4 Bioinformatic Analysis of miR-16 Target Genes

To better understand how APS affects chicken biological functions by modulating miR-16 expression, this study predicted its target genes using TargetScan and miRanda algorithms. The two algorithms identified 560 common target genes for subsequent functional enrichment analysis.

2.4.1 GO Enrichment Analysis of miR-16 Target Genes

GO enrichment analysis was performed on the 560 predicted target genes, with 229 genes receiving annotation. GO enrichment includes three aspects: biological process, cellular component, and molecular function. As shown in Table 2 , predicted target genes were extremely significantly enriched in biological processes including transmembrane transport, nucleotide metabolism, and notochord development ($P<0.01$), and significantly enriched in metabolism-related processes such as ubiquitin-dependent protein catabolic process, intracellular protein transport, glycolytic process, protein polyubiquitination, pentose-phosphate shunt, and negative regulation of protein ubiquitination ($P<0.05$). Immune-related pathways also showed enrichment ($P>0.05$), including Toll-like receptor (TLR) signaling pathways (TLR2, TLR3, TLR4, TLR5, TLR7, TLR15, TLR21) (GO:0002224, GO:0034134, GO:0034138, GO:0034142, GO:0034146, GO:0034154, GO:0035681, GO:0035682), negative regulation of interleukin-2 production (GO:0032703), negative regulation of interleukin-17 production (GO:0032700), negative regulation of interferon- production (GO:0032689), negative regulation of B cell differentiation (GO:0045578), and T cell differentiation (GO:0042098).

Cellular component analysis revealed that most miR-16 predicted target genes encode proteins located on the cell membrane (Table 3). Molecular function analysis showed extremely significant enrichment in hydrolase activity ($P<0.01$) (Table 4), with enrichment also observed in TLR binding (GO:0035325, $P=0.08$).

Table 2 GO term enrichment for the predicted target genes of miR-16 on biological process

Items	S gene number	TS gene number	B gene number	TB gene number	P-value
Transmembrane transport					<0.01

Items	S gene number	TS gene number	B gene number	TB gene number	P-value
Ubiquitin-dependent protein catabolic process					<0.05
Intracellular protein transport					<0.05
Glycolytic process					<0.05
Nucleotide metabolic process					<0.01
Protein polyubiquitination					<0.05
Neural tube closure					<0.05
Ureteric bud development					<0.05
Notochord development					<0.01
Response to pain					<0.05
Pentose-phosphate shunt					<0.05

Items	S gene number	TS gene number	B gene number	TB gene number	P-value
Negative regulation of protein ubiquitination					<0.05

Table 3 GO term enrichment for the predicted target genes of miR-16 on cellular component

Items	S gene number	TS gene number	B gene number	TB gene number	P-value
Membrane					
Nuclear membrane					
Ubiquitin ligase complex					
Autophagic vacuole					
Pre-autophagosomal structure membrane					

Table 4 GO term enrichment for the predicted target genes of miR-16 on molecular function

Items	S gene number	TS gene number	B gene number	TB gene number	P-value
Hydrolase activity					<0.01
Nucleic acid binding					
Ubiquitin-protein transferase activity					
Pancreatic ribonuclease activity					
5’ -nucleotidase activity					

2.4.2 KEGG Enrichment Analysis of miR-16 Target Genes

As shown in Table 5 , KEGG pathway analysis revealed that miR-16 predicted target genes are closely associated with cell motility, proliferation, differentiation, and material metabolism. Significantly enriched pathways included focal adhesion, insulin signaling pathway, ErbB signaling pathway, glycolysis/gluconeogenesis, valine/leucine/isoleucine biosynthesis, nicotinate and nicotinamide metabolism, fatty acid elongation, and DNA replication ($P<0.05$). Immune-related pathways also showed enrichment ($P>0.05$), primarily natural killer cell-mediated cytotoxicity (ko04650) and TLR signaling pathway (ko04620), which are associated with antigen recognition and presentation.

Table 5 Pathway analysis for the predicted target genes of miR-16

Pathway description	S gene number	TS gene number	B gene number	TB gene number	P-value
Glycolysis/Gluconeogenesis					<0.05
ErbB signaling pathway					<0.05

Pathway description	S gene number	TS gene number	B gene number	TB gene number	P-value
Focal adhesion					<0.05
Insulin signaling pathway					<0.05
Fatty acid elongation					<0.05
DNA replication					<0.05
Valine, leucine and isoleucine biosynthesis					<0.05
Nicotinate and nicotinamide metabolism					<0.05
GnRH signaling pathway					
Pentose phosphate pathway					
PPAR signaling pathway					
Galactose metabolism					
Endocytosis					
Cell cycle					

3.1 Effects of APS on Semen Quality of Breeder Cocks

APS is a functional polysaccharide extracted from traditional Chinese medicinal herb Astragalus membranaceus. Our previous studies demonstrated that dietary supplementation with 10 g/kg APS in breeder cocks significantly affected growth performance in commercial broiler offspring, with transcriptome sequencing data indicating that APS could regulate immune-related gene expression in breeder cocks and transgenerationally affect immune performance in progeny. Semen quality reflects animal reproductive performance and serves as an important production indicator, while spermatogenesis and sperm function represent the sole pathway for APS to exert transgenerational effects on broiler growth performance and immune function. Although the current results showed that APS did not significantly affect semen quality, it tended to improve semen characteristics by reducing sperm abnormality rate and increasing effective sperm number and semen volume. Additionally, Liu et al. reported that Astragalus

extract significantly improved sperm motility and effective sperm number in vitro, while Kim et al. found that APS effectively alleviated cyclophosphamide-induced reproductive toxicity in mice, improving testis relative weight and semen quality. Therefore, this study selected miR-16, which was differentially expressed in testis (the sole site of spermatogenesis) under APS supplementation, as the research target, combined with analysis of its tissue-specific expression patterns and bioinformatic analysis of target genes to provide clues for future investigations into APS-mediated regulation of metabolism and immune performance through miR-16.

3.2 Real-time Quantitative Detection of miRNA

miRNAs are trace, dynamic epigenetic regulatory factors. Analyzing miRNA expression patterns in different tissues under various nutritional states and identifying tissue-specific and functionally relevant miRNAs are critical steps for understanding miRNA function, with quantitative detection being the first step in miRNA functional studies. Current miRNA quantification methods include microarray chips, Northern blotting, high-throughput sequencing, and real-time quantitative PCR. Stem-loop real-time quantitative PCR offers advantages of high sensitivity, accuracy, and wide detection range. The customized stem-loop primers used in this study demonstrated good specificity and high sensitivity, meeting the requirements for accurate quantitative detection.

3.3 Effects of APS on miR-16 Expression in Different Tissues of Breeder Cocks

miRNA expression exhibits temporal and spatial specificity that is closely associated with particular physiological functions. Yue et al. reported that miR-16 was expressed in liver, spleen, kidney, small intestine, and testis of adult rats with tissue-specific differences. This study found that miR-16 expression showed tissue-specific differences in broiler breeder cocks, consistent with findings for many other miRNAs. Moreover, APS altered miR-16 expression in multiple tissues including spleen, liver, and testis. When the external environment changes, organisms undergo adaptive adjustments with altered gene expression levels, in which miRNAs play important regulatory roles by affecting target gene expression. Casas-Agustench et al. found that different fatty acid components regulated differential miRNA expression in rat liver and adipose tissue. Meale et al. reported that different fat components regulated differential miRNA expression in bovine subcutaneous and visceral adipose tissues. Romao et al. demonstrated that high-fat diet regulated differential miRNA expression in ovine subcutaneous and visceral tissues. These findings collectively indicate that different nutritional factors can regulate tissue-specific miRNA expression to affect metabolic regulation. In vitro studies showed that curcumin supplementation upregulated miR-16 expression, inducing changes in apoptosis and proliferation-related gene expression. Epigallocatechin gallate (EGCG), a green tea leaf extract, upregulated miR-16 expression, inhibited anti-apoptotic protein

BCL-2 gene expression, and activated apoptosis. Current miRNA research primarily focuses on associations among nutritional factors, miRNAs, and health status, with appropriate nutrition maintaining normal miRNA expression and healthy physiological states. As a potent plant-derived immunomodulator, APS may exert biological regulatory effects by influencing miR-16 expression across multiple tissues to regulate related gene expression and maintain health. Yuan et al. found that APS dose-dependently regulated TNF- and IL-1 expression in spleen. Under LPS stimulation, Liu et al. demonstrated that APS regulated intestinal mucosal IL-1 expression. Testis forms a complex immune network system through the blood-testis barrier, Sertoli cells, interstitial immune cells, cytokines, and androgens. Zhang et al. detected expression of TLR-2-I, -2-II, -3, -4, -5, -15, -21 in chicken testis, with LPS stimulation altering testicular IL-1 and IL-6 expression. Numerous studies have shown that APS regulates expression of cytokines including TNF- , IL-1 , IL-8, and IFN- , and miR-16 can regulate these cytokines through complementary binding to highly conserved regions, thereby affecting immune performance. Our results showed that APS upregulated miR-16 expression in duodenum mucosa, ileum mucosa, and spleen while downregulating it in testis, possibly regulating cytokine expression through miR-16 to influence immune performance. Additionally, APS can induce expression of the lipid metabolism-related gene PPAR- , and the PPAR signaling pathway containing PPAR- is an enriched pathway for miR-16 predicted target genes. The significant increase in hepatic miR-16 expression in APS-supplemented cobs suggests that miR-16 may mediate APS regulation of liver lipid metabolism-related genes.

3.4 Functional Prediction Analysis of miR-16 Target Genes

miRNAs exert biological regulatory functions by binding to the 3' UTR of target genes, leading to mRNA degradation or translational inhibition. This study found that APS significantly altered miR-16 expression levels in multiple tissues of breeder cocks. We therefore predicted miR-16 target genes using TargetScan and miRanda algorithms, using the intersection as predicted targets for GO and KEGG pathway enrichment analysis.

GO analysis revealed that predicted target genes were primarily enriched in biological processes related to material transport and metabolism, including transmembrane transport, ubiquitin-dependent protein degradation, intracellular protein transport, and glycolysis. KEGG enrichment results were consistent with GO analysis, with most target genes enriched in metabolism-related pathways, indicating that miR-16 interacts with metabolism-related genes to participate in organismal regulation.

miR-16 is closely associated with energy metabolism processes. Its predicted target genes were enriched in glycolysis/gluconeogenesis, a key component of energy metabolism. The immune activity regulated by APS also requires substantial energy consumption, which is intimately linked to energy metabolism. Calin et al. found that the miR-15a/miR-16-1 cluster was significantly downregulated

or absent in chronic lymphocytic leukemia, markedly reducing activity of the glycolytic enzyme aldolase A (ALDOA). The insulin signaling pathway broadly affects glucose uptake, synthesis of the three major nutrients, cell proliferation, differentiation, and apoptosis. Glucose transporter 4 (GLUT4) is an important regulator of the insulin signaling pathway, mediating glucose uptake from extracellular to intracellular compartments and regulating intracellular glucose and fatty acid metabolism. Talari et al. found that miR-16 overexpression in myoblasts enhanced insulin-mediated glucose uptake by upregulating GLUT4, thereby affecting cellular glucose metabolism. Exogenous APS also affects energy metabolism, as Chen et al. reported that APS induced high GLUT4 expression while inhibiting PPAR- and its target genes fatty acid transport protein (FATP) and acyl-CoA synthetase (ACS), thus influencing energy metabolism. Our study found that APS regulated miR-16 expression in liver, the central metabolic organ, and previous studies have shown miR-16 is closely related to energy metabolism, suggesting APS may affect energy metabolism by regulating miR-16 expression. Additionally, as the intestine is an important organ for digestion and absorption, APS upregulated miR-16 expression in duodenum and ileum mucosa, and its target genes were enriched in pathways related to material transport and metabolism such as transmembrane transport, intracellular protein transport, and glycolysis, indicating APS may affect material metabolism by regulating miR-16 expression to influence material transport.

APS significantly increased miR-16 expression in spleen, duodenum mucosa, and ileum mucosa of breeder cocks. Studies have reported that miR-16 may be an important regulator of TLR-mediated inflammatory responses, as it can rapidly degrade mRNAs containing AU-rich elements (AREs) in the 3' UTR region. Most cytokines and chemokines, including TNF- , IL-8, and IL-6, contain AREs in their 3' UTR regions. Jing et al. confirmed that miR-16 can directly bind to the AREs of TNF- mRNA, reducing mRNA stability and promoting its degradation, an effect reversible by transfection with sequence-specific complementary oligonucleotides. Further analysis revealed that IL-12p40, the common subunit of inflammatory factors IL-12 and IL-23, also contains AREs in its 3' UTR region, and miR-16 can bind to AREs of both TNF- and IL-12p40 to regulate expression of numerous inflammatory factors including TNF- , IL-12, IL-23, IL-17, IFN- , IL-1 , and IL-6. Additionally, miR-16 can inhibit LPS-induced inflammatory responses by downregulating TLR4 and IL-1 receptor-associated kinase transcriptional activity. In this study, miR-16 predicted target genes were enriched in immune-related GO terms and KEGG pathways, indicating close association between miR-16 and immune regulation. Combined with previous studies, this suggests miR-16 possesses anti-inflammatory activity to maintain normal health status. Moreover, APS exhibits high immunomodulatory activity, affecting immune function in spleen and intestinal mucosal immune system. Shi et al. found that APS effectively improved mucosal integrity and immune function, antagonized LPS-induced elevation of IL-1 and IFN- expression, and prevented inflammation while promoting jejunal mucosal integrity. Wang et al. reported that APS effectively inhibited expression of pro-inflammatory cy-

tokines (TNF- , IL-1 , and IL-8) in LPS-treated Caco2 cells and increased expression of tight junction proteins and occludin, exerting anti-inflammatory effects and protecting mucosal integrity. Our study found that APS significantly increased miR-16 expression in duodenum mucosa, ileum mucosa, and spleen of breeder cocks, suggesting APS may exert its immunomodulatory functions in intestinal mucosa and spleen by upregulating miR-16.

In conclusion, miR-16 is differentially expressed in various tissues of breeder cocks. APS upregulates miR-16 expression in spleen, liver, duodenum mucosa, and ileum mucosa while downregulating it in testis. Functional prediction reveals that miR-16 target genes are closely associated with material metabolism and immune regulation, indicating that APS affects metabolic and immune regulatory functions by modulating miR-16 expression.

References

[1] CHEN W, XIA Y P, CHEN W J, et al. Improvement of myocardial glycolipid metabolic disorder in diabetic hamster with Astragalus polysaccharides treatment[J]. Molecular Biology Reports, 2012, 39(7): 7609-7615.

[2] HE X J, SHU J, XU L, et al. Inhibitory effect of Astragalus polysaccharides on lipopolysaccharide-induced TNF- and IL-1 production in THP-1 cells[J]. Molecules, 2012, 17(3): 3155-3164.

[3] WANG X F, LI Y L, YANG X J, et al. Astragalus polysaccharide reduces inflammatory response by decreasing permeability of LPS-infected Caco2 cells[J]. International Journal of Biological Macromolecules, 2013, 61: 347-352.

[4] ZHANG G G, YANG Z B, WANG Y, et al. Effects of Astragalus membranaceus root processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens[J]. Poultry Science, 2013, 92(1): 178-183.

[5] ZUO Z Y, YANG W R, WANG Y, et al. Effects of Astragalus membranaceus on laying performance and antioxidant status of laying hens[J]. The Journal of Applied Poultry Research, 2012, 21(2): 243-250.

[6] ZHU Z Y, LIU R Q, SI C L, et al. Structural analysis and anti-tumor activity comparison of polysaccharides from Astragalus[J]. Carbohydrate Polymers, 2011, 85(4): 895-902.

[7] SUN S Y, ZHENG K, ZHAO H Y, et al. Regulatory effect of Astragalus polysaccharides on intestinal intraepithelial T cells of tumor bearing mice[J]. Molecules, 2014, 19(9): 15224-15236.

[8] ABUELSAAD A S A. Supplementation with Astragalus polysaccharides alters Aeromonas-induced tissue-specific cellular immune response[J]. Microbial Pathogenesis, 2014, 66: 48-56.

[9] GUIL S, ESTELLER M. RNA-RNA interactions in gene regulation: the

coding and noncoding players[J]. Trends in Biochemical Sciences, 2015, 40(5): 248–256.

[10] ROSS S A, DAVIS C D. The emerging role of microRNAs and nutrition in modulating health and disease[J]. Annual Review of Nutrition, 2014, 34(1): 305–336.

[11] LIANG Y, RIDZON D, WONG L, et al. Characterization of microRNA expression profiles in normal human tissues[J]. BMC Genomics, 2007, 8(1): 166–185.

[12] BUSHATI N, COHEN S M. microRNA functions[J]. Annual Review of Cell and Developmental Biology, 2007, 23(1): 175–205.

[13] XIAO C C, RAJEWSKY K. MicroRNA control in the immune system: basic principles[J]. Cell, 2009, 136(1): 26–36.

[14] TÜFEKCI K U, MEUWISSEN R L J, GENÇ Ş. The role of microRNAs in biological processes[M]//YOUSEF M, ALLMER J. miRNomics: MicroRNA Biology and Computational Analysis. New York: Humana Press, 2014: 15–31.

[15] LEE H M, NGUYEN D T, LU L F. Progress and challenge of microRNA research in immunity[J]. Frontiers in Genetics, 2014, 5: 178.

[16] KAPPIL M, CHEN J. Environmental influence of microRNA in Children' s health[J]. Current Opinion in Pediatrics, 2014, 26(2): 243–251.

[17] HARRYANTO H. Role of MicroRNA in early life placental programming of insulin resistance and metabolic health[D]. Ph.D. Thesis. Adelaide: The University of Adelaide, 2014.

[18] MOBARRA N, SHAFIEE A, RAD S M A H, et al. Overexpression of microRNA-16 declines cellular growth, proliferation and induces apoptosis in human breast cancer cells[J]. In Vitro Cellular & Developmental Biology-Animal, 2015, 51(6): 604–611.

[19] YANG T Q, LU X J, WU T F, et al. MicroRNA-16 inhibits glioma cell growth and invasion through suppression of BCL2 and the nuclear factor- B1/MMP9 signaling pathway[J]. Cancer Science, 2014, 105(3): 265–271.

[20] HUANG E Y, LIU R H, CHU Y W. miRNA-15a/16: as tumor suppressors and more[J]. Future Oncology, 2015, 11(16): 2351–2363.

[21] ZHOU R, LI X Q, HU G K, et al. miR-16 targets transcriptional corepressor SMRT and modulates NF-kappaB-regulated transactivation of interleukin-8 gene[J]. PLoS One, 2012, 7(1): e30772.

[22] LI T, MORGAN M J, CHOKSI S, et al. MicroRNAs modulate the noncanonical transcription factor NF- B pathway by regulating expression of the kinase IKK during macrophage differentiation[J]. Nature Immunology, 2010, 11(9): 799–805.

[23] CALIN G A, CIMMINO A, FABBRI M, et al. MiR-15a and miR-16-1 cluster functions in human leukemia[J]. Proceedings of the National Academy of Sciences of the United States of America, 2008, 105(13): 5166–5171.

[24] TALARI M, KAPADIA B, KAIN V, et al. MicroRNA-16 modulates macrophage polarization leading to improved insulin sensitivity in myoblasts[J]. Biochimie, 2015, 119: 16–26.

[25] LI Yulong. Transgenerational transcriptional analysis of APS-regulated genes in breeder cocks[D]. Master' s Thesis. Yangling: Northwest A&F University, 2015.

[26] LIU J, LIANG P, YIN C, et al. Effects of several Chinese herbal aqueous extracts on human sperm motility in vitro[J]. Andrologia, 2004, 36(2): 78–83.

[27] KIM W, KIM S H, PARK S K, et al. Astragalus membranaceus ameliorates reproductive toxicity induced by cyclophosphamide in mice[J]. Phytotherapy Research, 2012, 26(9): 1418–1421.

[28] CHEN C F, RIDZON D A, BROOMER A J, et al. Real-time quantification of microRNAs by stem-loop RT-PCR[J]. Nucleic Acids Research, 2005, 33(20): e179.

[29] SMALL E M, OLSON E N. Pervasive roles of microRNAs in cardiovascular biology[J]. Nature, 2011, 469(7330): 336–342.

[30] YUE J, TIGYI G. Conservation of miR-15a/16-1 and miR-15b/16-2 clusters[J]. Mammalian Genome, 2010, 21(1/2): 88–94.

[31] LIANG Y, RIDZON D, WONG L, et al. Characterization of microRNA expression profiles in normal human tissues[J]. BMC Genomics, 2007, 8(1): 166.

[32] CASAS-AGUSTENCH P, FERNANDES F S, TAVARES DO CARMO M G, et al. Consumption of distinct dietary lipids during early pregnancy differentially modulates the expression of microRNAs in mothers and offspring[J]. PLoS One, 2015, 10(2): e0117858.

[33] MEALE S J, ROMAO J M, HE M L, et al. Effect of diet on microRNA expression in ovine subcutaneous and visceral adipose tissues[J]. Journal of Animal Science, 2014, 92(8): 3328–3337.

[34] ROMAO J M, JIN W, HE M L, et al. Altered microRNA expression in bovine subcutaneous and visceral adipose tissues from cattle under different diet[J]. PLoS One, 2012, 7(7): e40605.

[35] YANG J, CAO Y X, SUN J F, et al. Curcumin reduces the expression of Bcl-2 by upregulating miR-15a and miR-16 in MCF-7 cells[J]. Medical Oncology, 2010, 27(4): 1114–1118.

[36] GAO S M, YANG J J, CHEN C Q, et al. Pure curcumin decreases the expression of WT1 by upregulation of miR-15a and miR-16-1 in leukemic cells[J]. Journal of Experimental & Clinical Cancer Research, 2012, 31(1): 27.

[37] TSANG W P, KWOK T T. Epigallocatechin gallate up-regulation of miR-16 and induction of apoptosis in human cancer cells[J]. The Journal of Nutritional Biochemistry, 2010, 21(2): 140–146.

[38] YUAN C T, PAN X P, GONG Y, et al. Effects of Astragalus polysaccharides (APS) on the expression of immune response genes in head kidney, gill and spleen of the common carp, Cyprinus carpio L.[J]. International Immunopharmacology, 2008, 8(1): 51–58.

[39] LIU L, SHEN J, ZHAO C, et al. Dietary Astragalus polysaccharide alleviates immunological stress in broilers exposed to lipopolysaccharide[J]. International Journal of Biological Macromolecules, 2015, 72: 624–632.

[40] ZHANG M, NII T, ISOBE N, et al. Expression of Toll-like receptors and effects of lipopolysaccharide on the expression of proinflammatory cytokines and chemokine in the testis and epididymis of roosters[J]. Poultry Science, 2012, 91(8): 1997–2003.

[41] ZHANG C L, REN H J, LIU M M, et al. Modulation of intestinal epithelial cell proliferation, migration, and differentiation in vitro by Astragalus polysaccharides[J]. PLoS One, 2014, 9(8): e106674.

[42] WANG X F, SHEN J, LI S Z, et al. Sulfated Astragalus polysaccharide regulates the inflammatory reaction in LPS-infected broiler chicks[J]. International Journal of Biological Macromolecules, 2014, 69: 146–150.

[43] WANG X F, WANG S Y, LI Y L, et al. Sulfated Astragalus polysaccharide can regulate the inflammatory reaction induced by LPS in Caco2 cells[J]. International Journal of Biological Macromolecules, 2013, 60: 248–252.

[44] SHI J J, ZHAO Y, WANG Y P, et al. Inflammatory caspases are innate immune receptors for intracellular LPS[J]. Nature, 2014, 514(7521): 187–192.

[45] BARTEL D P. MicroRNAs: target recognition and regulatory functions[J]. Cell, 2009, 136(2): 215–233.

[46] SALTIEL A R, PESSIN J E. Insulin signaling pathways in time and space[J]. Trends in Cell Biology, 2002, 12(2): 65–71.

[47] JING Q, HUANG S, GUTH S, et al. Involvement of microRNA in AU-rich element-mediated mRNA instability[J]. Cell, 2005, 120(5): 623–634.

[48] ANDERSON P. Post-transcriptional control of cytokine production[J]. Nature Immunology, 2008, 9(4): 353–359.

[49] HUANG Z, MA J T, CHEN M J, et al. Dual TNF- /IL-12p40 interference as a strategy to protect against colitis based on miR-16 precursors with macrophage targeting vectors[J]. Molecular Therapy, 2015, 23(10): 1611–1621.

[50] WANG X L, WANG X L, LIU X L, et al. miR-15a/16 are upregulated in the serum of neonatal sepsis patients and inhibit the LPS-induced inflammatory

pathway[J]. International Journal of Clinical and Experimental Medicine, 2015, 8(4): 5683-5690.

[51] ZHANG X H, WANG D Y, HU Y L, et al. Immunologic enhancement of Astragalus polysaccharide (APS) on the humoral immunity of chicken[J]. Chinese Journal of Veterinary Science, 2009, 29(3): 312-314, 334.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.