Expression profiles of novel genes and microRNAs involved in lipid deposition in chicken’s adipocyte

HuaYun Huanga,b, GuiPing Zhaoa, RanRan Liu, ShouFeng Lib, ZhenHua Zhaoaab, QingHe Lia, MaiQing Zhenga and Jie Wena

aInstitute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China; bInstitute of Poultry Science, Chinese Academy of Agriculture Sciences, Yangzhou, P. R. China

ABSTRACT

With the aim of studying the molecular mechanisms underlying lipid deposition in chickens, the expression profiles of eight novel candidate genes and miRNA involved in lipid deposition were examined in the undifferentiated and differentiated adipocyte at 3d, 5d and 10d after inducing differentiation. The level of expression of gga-miR-30 cluster (gga-miR-30a-5p, miR-30c-5p and miR-30e-5p) and miR-17-92 cluster (gga-miR-17-5p, miR-19a-3b and miR-20a-5p), compared to undifferentiated adipocyte, was significantly up-regulated with differentiated adipocyte; expression of miR-103-3p and miR-92-5p was significantly up-regulated with differentiated adipocyte. Seven genes, ACSL1, CYB5A, SEC23A, BRP44L, PLTP, MGLL and GART, compared to undifferentiated adipocyte, were significantly down-regulated with differentiated adipocyte. Correlation analysis of the detected genes and miRNAs indicated BRP44L may be a target of miR-103-3p and it was negatively correlated with miR-103-3p. CYB5A may be a target of miR-30a-5p and it was negatively correlated with miR-30a-5p. SEC23A may be a target of miR-19a-3p and it was negatively correlated with miR-19a-3p. MGLL may be a target of miR-19a-3p and it was negatively correlated with miR-19a-3p. These findings will improve more integrated information of the miRNA and genes in regulating the quantity of lipid deposition in chicken adipocyte.

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Introduction

Excessive fat accretion is a crucial problem during broiler production. The pathological and physiological fat deposition is regulated by genetic and epigenetic factors (Shi et al. 2011; Heitmann et al. 2012). Some genes and transcription factors were validated and associated with fat deposition in chickens (Gondret et al. 2001; Larkina et al. 2011; Shi et al. 2011; Huang et al. 2014). Lipid deposition was commonly regulated by multiple genes, it should be feasible to find more candidate genes and further understand its molecular genetic controls.

MicroRNAs (miRNAs) are endogenous, non-coding small regulatory RNAs of 19 to 24 nucleotides (nt) that bind to complementary target sites in the 3’ untranslated region (UTR) of mRNAs, resulting in translational repression and/or mRNA destabilisation (Carrington and Ambros 2003; Bartel 2004). These small regulatory RNAs have been implicated in lipogenesis (Kajimoto et al. 2006; Nakanishi et al. 2009; Ling et al. 2011).

So far, many genes and miRNA were detected by microarray and deep sequencing in chicken adipocyte and abdominal fat tissues (Wang et al. 2013; Huang et al. 2015). However, little about these genes and miRNA were further studied. In the present study, the pattern of some genes, ACSL1, SOCS3, MGLL, BRP44L, CYB5A, PLTP, SEC23A and GART, and miRNA, gga-miR-17-5p, miR-19a-3b, miR-20a-5p, miR-19a-3p, miR-20a-5p, gga-miR-30a-5p, miR-30c-5p and miR-30e-5p, based on previous differentially expressed miRNAs and differentially expressed genes in a high-abdominal-fat chicken (HAbF) and low abdominal-fat group (LAbF) (Huang et al. 2015), were detected in undifferentiated and differentiated adipocyte and obtained some novel candidate genes and miRNA related to lipid deposition.

Material and methods

Culture of preadipocytes

All the animal experiments were done in accordance with the Guidelines for Experimental Animals.

CONTACT

Professor Jie Wen wenjie@caas.cn Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R. China

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established by the Ministry of Science and Technology (Beijing, China). Ethical approval on animal survival was given by the animal ethics committee of Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (IAS-CAAS) with the following reference number: IASCAAS-AE-03. The chickens (Beijing-You) were raised under the same recommended environmental and nutritional conditions at the conservation farm of the IAS, CAAS. Preadipocytes were isolated from the abdominal adipose tissue from female chickens (age, 2–4 weeks) following methods described previously (Ramsay and Rosebrough 2003; Liu et al. 2009).

Cells were cultured in six-well culture dishes. The culture medium was DMEM/F12 (1:1) containing 10% FBS and 1% penicillin/streptomycin solution at 37°C in a humidified atmosphere of 5% CO2 in air. Cells reached 70% confluence, then differentiation was induced with monocyte differentiation-inducing factors (MDI, 3-isobutyl-1-methylxanthine, 0.5 mmol/L; dexamethasone, 1 μmol/L; insulin, 1 mg/L) for 2 days followed by another 2 days with only insulin (1 mg/L) added to the basic culture medium. Cells of were harvested at 0 (cells reached 80% confluence and undifferentiated group), 3, 5 and 10 d after inducing differentiation. Each group was in triplicate.

RNA extraction

Total RNA was isolated from preadipocytes using a commercially available kit according to the manufacturer’s protocol (DP419, Tiangen, Beijing, China). The concentration and purity of RNAs were determined by A260 and A260:280 (A260:280 ≥ 1.8 and ≤ 2.0) using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). RNA samples were stored at −80°C until used.

Quantitative real-time PCR of mRNA and miRNA

The relative expression profiles of eight genes involved in lipid deposition in undifferentiated and differentiated fat cell were identified in adipocyte using the Quantifast SYBR Green PCR Kit (Qiagen, Düsseldorf, Germany). The final concentration of each primer was 10μmol/μL. GAPDH was chosen as an internal control to correct for analytical variations. The primers used are described in Table S1.

Parallel quantitative real-time PCRs (qPCRs) were used to quantify relevant miRNAs, also using Qiagen’s methodology (the miScript II RT Kit and the miScript SYBR Green PCR Kit) with a miRNA-specific forward primer (Table S2) and the universal reverse primer provided. U6 was chosen as an internal control to correct for analytical variations.

Target genes prediction

The target genes of the miRNAs were predicted on the basis of chicken sequences using the TargetScan (http://www.targetscan.org) and miRanda (http://cbio.mskcc.org/miRNA2003/miranda.html) algorithms.

Statistical methods

The expression data were analysed with the ANOVA procedure (Duncan’s tests) of SPSS 16.0 to assess differences of different stages in adipocyte. Correlation analysis was conducted using Pearson of Bivariate correlation. A p-value less than .05 was considered significant.

Results

The expression pattern of genes in undifferentiated and differentiated adipocyte

The expression pattern of eight genes, ACsL1, SOCS3, MGLL, BRP44L, CYB5A, PLTP, SEC23A and GART, were detected using qPCR in undifferentiated and differentiated fat cell. The results showed that, with the exception of SOCS3, the level expression of 7 genes in undifferentiated fat cell was significantly higher than that of in differentiated cell (p < .05). The expression of 4 genes (ACsL1, MGLL, BRP44L, SEC23A and GART) was the lowest level after inducing differentiation 3d (Figure 1). However, the level of expression of SOCS3 mRNA was the highest after inducing differentiation 3d (Figure 1).

The pattern of miRNA in undifferentiated and differentiated adipocyte

Expression of three miRNA (gga-miR-17-5p, miR-19a-3p and miR-20a-5p), belonged to miR17-92 cluster, were detected by qPCR in undifferentiated and differentiated adipocyte. The results indicated the expression of three miRNA in differentiated fat cell was significantly higher than that of in undifferentiated cell (p < .05). The expression of 4 genes (ACsL1, MGLL, BRP44L, SEC23A and GART) was the lowest level after inducing differentiation 3d (Figure 1). However, the level of expression of SOCS3 mRNA was the highest after inducing differentiation 3d (Figure 1).
with time and peaked at 10d after inducing differentiation.

Expression profiles of three miRNA, gga-miR-30a-5p, miR-30c-5p and miR-30e-5p, belonged to miR-30 cluster, were detected by qPCR in undifferentiated and differentiated adipocyte. The results indicated, compared with the undifferentiated cell group, was up-regulated with differentiated adipocyte (Figure 2(b), p < .05), but expression profiles of three miRNA is slight different. miR-30a-5p expression peaked at 3d after inducing-differentiation, miR-30c-5p and miR-30e-5p peaked at 5d after inducing differentiation.

Expression of gga-miR-92-3p and miR-103-3p in differentiated fat cell were significantly higher than that in undifferentiated cell (Figure 2(a,b), p < .05) and peaked at 3d after inducing differentiation.

Integrated analysis of genes and miRNA

It is well known that miRNA regulated target traits by binding to complementary target sites in the 3’UTR of mRNAs. One miRNA will have one or more target genes, one gene will be regulated by one or more miRNAs.

From the analysis using the TargetScan and miRanda algorithms, BRP44L transcripts may be a target of miR-103-3p (Figure S1-A) and miR-30c-5p (Figure S1-B); CYB5A may be a target of miR-19a-3p (Figure S1-C) and miR-30a-5p (Figure S1-D); SEC23A may be a target of miR-19a-3p (Figure S1-E) and miR-30c-5p (Figure S1-F); MGLL may be a target of miR-19a-3p (Figure S1-G) and miR-92a-5p (Figure S1-H).

Correlation of miRNA expression with target genes

Changes in the expression of BRP44L were high negatively correlated with miR-103-3p (r = -0.831, p = .001), CYB5A was high negatively correlated with miR-30a-5p (r = -0.865, p = .000). The expression of SEC23A was moderate negatively correlated with miR-19a-3p (r = -0.753, p = .005). MGLL was high negatively correlated with miR-19a-3p (r = -0.887, p = .000) and miR-92a-5p (r = -0.618, p = .032).

Discussion

Genes and lipid deposition in chicken

Five genes (ACSL1, SOCS3, MGLL, PLTP and CYB5A) known to have direct or indirect association with lipid metabolism in mammals. ACSL1 influenced lipolysis and β-oxidation rates in 3T3-L1 adipocytes (Ellis et al. 2010). MGLL completes the hydrolysis of intracellular triglyceride stores and lipoprotein-derived triglycerides (Karlsson et al. 2001). Cytochrome b5 (CYB5A) plays the key role in the formation of lipid-radical cycles (Agadzhanyan et al. 2013). SOCS3 promotes adipocyte apoptosis by both aggravating inflammation and inhibiting the activity of JAK2/STAT3 signalling pathway in 3T3-L1 cell line (Liu et al. 2015). Adipocyte PLTP plays a small but significant role in plasma PLTP activity and promotes cholesterol efflux from adipose tissues (Jiang et al. 2015). Additional genes exposed here include GART, SEC23A and BRP44L and if or how they affect lipid metabolism or fat accumulation is not apparent.

No previous studies have associated ACSL1, SOCS3, MGLL, PLTP, CYB5A, GART, SEC23A and BRP44L with...
lipid deposition in chicken. Our team group identified that they displayed significant differential expression in HAbF and LAbF chicken (Huang et al. 2015). On the grounds that they displayed significant differential expression here, further studies of these genes seem to be warranted. Although detailed functional characterisation of these eight genes in chicken lipid metabolism is lacking, the present findings, combined with our previous studies, suggest that these genes may play key roles in the regulation of lipid deposition in chicken adipocyte.

**miRNA and lipid deposition in chicken**

The miR17-92 cluster comprises seven miRNAs (miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92-1) and it accelerate adipocyte differentiation by negatively regulating the tumour-suppressor Rb2/p130 (Wang et al. 2008). Three miRNA (ggamir-17-5p, miR-19a and miR-20a) identified by deep sequencing were differentially expressed (Huang et al. 2015). So expression profiles of three miRNA were further studied in adipocyte. It is noteworthy that three miRNA is up-regulated with differentiated adipocyte, which was consistent with our previous studies in AbF chicken. Taken together, these findings indicate that these three miRNAs may play important roles in the regulation of lipid deposition in chickens.

The miR-30 cluster comprises five miRNAs (miR-30a, miR-30b, miR-30c, miR-30d and miR-30e). In 3T3-L1 cell, miR-30e is indispensable for maintaining the balance of adipocytes by targeting the canonical Wnt/β-catenin signalling (Wang et al. 2013). Over-expression of hepatic miR-30c curtails hyperlipidaemia and atherosclerosis by decreasing lipid biosynthesis and lipoprotein secretion (Irani and Hussain 2015). No

![Figure 2. Expression profile of candidate miRNA in undifferentiated and differentiated adipocyte. Different superscripts indicate significant differences (p < .05 or p < .01) among stages. Un-DIF notes undifferentiated adipocyte; DIF notes differentiated adipocyte. gga: Gallus gallus.](image)
previous studies have associated miR-30 cluster with lipid metabolism, specifically in chickens. The present findings suggest that miR-30 cluster may play important regulatory roles in lipid deposition in chicken adipocyte.

**Conclusions**

In summary, eight miRNA, gga-miR-17-5p, miR-19a-3p, miR-20a-5p, miR-30a-5p, miR-30c-5p, miR-30e-5p, miR-103-3p and miR-92a-5p, and eight genes, ACSL1, SOCS3, MGLL, PLTP, CYB5A, GART, SEC23A and BRP44L, may play important regulatory roles in lipid deposition in chicken adipocyte. These miRNA and genes could be novel genes and miRNAs involved in lipid deposition in chicken’s adipocyte.

**Disclosure statement**

The authors declare no competing financial interests.

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**References**

Agadzhanyan ZS, Doroshchuk AD, Shiryaeva YK, Dmitriev LF. 2013. Role of cytochrome b5 and alpha-tocopherol to microsomal and mitochondrial oxidation. Bull Exp Biol Med. 156:191–195.

Bartel DP. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 116:281–297.

Carrington JC, Ambros V. 2003. Role of microRNAs in plant and animal development. Science. 301:336–338.

Ellis JM, Li LO, Wu PC, Koves TR, Ilkayeva O, Stevens RD, Watkins SM, Muoio DM, Coleman RA. 2010. Adipose acyl-CoA synthetase-1 directs fatty acids toward beta-oxidation and is required for cold thermogenesis. Cell Metab. 12:53–64.

Gondret F, Ferre P, Dugail I. 2001. ADD-1/5REBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. J Lipid Res. 42:106–113.

Heitmann BL, Westerterp KR, Loos RJ, Sorensen TI, O’Dea K, McLean P, Jensen TK, Eisenmann J, Speakman JR, Simpson SJ. 2012. Obesity: lessons from evolution and the environment. Obesity Rev. 13:910–922.

Huang AX, Li JJ, Tian Y, Shen JD, Tao ZR, Li GQ, Lu LZ, Fu Y, Wu TX. 2014. RNA interference of leptin receptor gene in chicken adipocytes. Genet Mol Res. 13:5901–5907.

Huang HY, Liu RR, Zhao GP, Li QH, Zheng MQ, Zhang JJ, Li SF, Liang Z, Wen J. 2015. Integrated analysis of microRNA and mRNA expression profiles in abdominal adipose tissues in chickens. Sci Rep. 5:16132.

Irani S, Hussain MM. 2015. Role of microRNA-30c in lipid metabolism, adipogenesis, cardiac remodeling and cancer. Curr Opin Lipidol. 26:139–146.

Jiang H, Yazdanyar A, Lou B, Chen Y, Zhao X, Li R, Hoang Bui H, Kuo MS, Navab M, Qin S, et al. 2015. Adipocyte phospholipid transfer protein and lipoprotein metabolism. Arteriosclerosis Thrombosis Vasc Biol. 35:316–322.

Kajimoto K, Naraba H, Iwai N. 2006. MicroRNA and 3T3-L1 pre-adipocyte differentiation. RNA (New York, NY). 12:1626–1632.

Karlsson M, Reue K, Xia YR, Lusis AJ, Langin D, Tornqvist H, Holm C. 2001. Exon-intron organization and chromosomal localization of the mouse monoglyceride lipase gene. Gene. 272:11–18.

Larkina TA, Sazanov AA, Fomichev KA, Barkova OY, Sazanov FA, Malewski T, Jaszczak K. 2011. [Expression profiling of candidate genes for abdominal fat mass in domestic chicken Gallus gallus]. Russ J Genet. 47:1140–1144.

Ling HY, Wen GB, Feng SD, Tuo QH, Ou HS, Yao CH, Zhu BY, Gao ZP, Zhang L, Liao DF. 2011. MicroRNA-375 promotes 3T3-L1 adipocyte differentiation through modulation of extracellular signal-regulated kinase signalling. Clin Exp Pharmacol Physiol. 38:239–246.

Liu S, Wang L, Wang N, Wang Y, Shi H, Li H. 2009. Oleate induces transdifferentiation of chicken fibroblasts into adipocyte-like cells. Comp Biochem Physiol A Mol Integr Physiol. 154:135–141.

Liu Z, Gan L, Zhou Z, Jin W, Sun C. 2015. SOCS3 promotes inflammation and apoptosis via inhibiting JAK2/STAT3 signalling pathway in 3T3-L1 adipocyte. Immunobiology. 220:947–953.

Nakanishi N, Nakagawa Y, Tokushige N, Aoki N, Matsuzaka T, Ishii K, Yahagi N, Kobayashi K, Yato S, Takahashi A, et al. 2009. The up-regulation of microRNA-335 is associated with lipid metabolism in liver and white adipose tissue of...
genetically obese mice. Biochem Biophys Res Commun. 385:492–496.

Ramsay TG, Rosebrough RW. 2003. Hormonal regulation of postnatal chicken preadipocyte differentiation in vitro. Comparative Biochem Physiol Part B, Biochem Mol Biol. 136:245–253.

Shi H, Zhang Q, Wang Y, Yang P, Wang Q, Li H. 2011. Chicken adipocyte fatty acid-binding protein knockdown affects expression of peroxisome proliferator-activated receptor gamma gene during oleate-induced adipocyte differentiation. Poultry Sci. 90:1037–1044.

Wang J, Guan X, Guo F, Zhou J, Chang A, Sun B, Cai Y, Ma Z, Dai C, Li X, et al. 2013. miR-30e reciprocally regulates the differentiation of adipocytes and osteoblasts by directly targeting low-density lipoprotein receptor-related protein 6. Cell Death Dis. 4:e845.

Wang Q, Li YC, Wang J, Kong J, Qi Y, Quigg RJ, Li X. 2008. miR-17-92 cluster accelerates adipocyte differentiation by negatively regulating tumor-suppressor Rb2/p130. Proc Natl Acad Sci USA. 105:2889–2894.

Wang W, Du ZQ, Cheng B, Wang Y, Yao J, Li Y, Cao Z, Luan P, Wang N, Li H. 2015. Expression profiling of preadipocyte microRNAs by deep sequencing on chicken lines divergently selected for abdominal fatness. PLoS One. 10:e0117843.

Yao J, Wang Y, Wang W, Wang N, Li H. 2011. Solexa sequencing analysis of chicken pre-adipocyte microRNAs. Biosci Biotechnol Biochem. 75:54–61.