Research in Veterinary Science 99 (2015) 99–104

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Differential miRNA expression profiles in the *longissimus dorsi* muscle between intact and castrated male pigs

Zhaowei Cai [a,b], Lifan Zhang [c], Xiaoling Jiang [a], Yifei Sheng [a], Ningying Xu [a,*]

[a] College of Animal Science, Zhejiang University, Hangzhou 310058, China
[b] Laboratory Animal Research Center, Zhejiang Chinese Medical University, Hangzhou 310053, China
[c] College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

ARTICLE INFO

Article history:
Received 11 April 2014
Accepted 17 December 2014

Keywords:
Castration
MicroRNA
Expression
Skeletal muscle
Pig

ABSTRACT

MicroRNAs (miRNAs) are important modulators of skeletal muscle development in multiple mammalian species, but their role in skeletal muscle growth in castrated male pigs has not been well studied. The aim of the present study was to determine the role of miRNAs in longissimus dorsi muscle under castration. Our results showed that castration caused a significant decrease in serum testosterone levels as well as carcass lean mass, but led to an increase in carcass fat mass. Moreover, miRNA expression profiles in skeletal muscle were significantly altered by castration, and seven differentially expressed miRNAs were discovered. More importantly, functional analysis suggested that these differentially expressed miRNAs and their targets are involved in the regulation of skeletal muscle contractile function and fat metabolism. Taken together, these results demonstrate altered miRNA expression in skeletal muscle of castrated male pigs, and suggest a potential mechanism underlying the effects of castration on porcine skeletal muscle growth.

1. Introduction

Castration of male pigs has been considered a traditional practice in most countries, and is performed to prevent boar taint produced and aggressive behavior shown by male animals (Sutherland et al., 2010). However, some disadvantages of castration, such as lower skeletal muscle mass and higher fat deposition, are also evident in the pig industry (Gispert et al., 2010). It is well known that castration of male pigs results in negative effects on skeletal muscle growth, but, thus far, the molecular basis for this defect is not clear.

MicroRNAs (miRNAs) are a class of small non-coding RNA transcripts that regulate gene expression by binding to genomic regions of specific target genes (von Deetzen et al., 2013). The role of miRNAs in the regulation of skeletal muscle growth and development has been previously demonstrated. For example, miR-26a is induced during skeletal muscle regeneration, and exogenous miR-26a promotes differentiation of myoblasts in humans (Dey et al., 2012); miR-27a increases muscle cell proliferation by directly inhibiting myostatin gene expression (Huang et al., 2012); miR-378 is considered a candidate for regulating myogenesis and participates in skeletal muscle development through the regulation of *BMP2* and *MAPK1* (Hou et al., 2012); and miR-155 affects porcine prenatal skeletal muscle development through the regulation of *OLFML3* (Zhao et al., 2012). Until now, little was known about the roles of miRNAs in the regulation of skeletal muscle growth and development in castrated male pigs. Specifically, it was not clear whether testosterone loss due to castration could influence miRNA expression patterns in porcine skeletal muscle. Previous studies have suggested that androgen treatment could alter miRNA expression levels in human skeletal muscle myoblasts. Androgen receptor (AR) regulates the downstream mRNA targets of these miRNAs specifically in skeletal muscle (Wyce et al., 2010). Moreover, androgen treatment upregulates the expression of a large set of miRNAs in the prostate and muscle in rats (Narayanan et al., 2010). We hypothesized that reduction of androgen levels due to castration may influence miRNA expression in skeletal muscle of male pigs. Therefore, we investigated the changes in miRNA expression profiles in the longissimus dorsi (LD) muscle between intact and castrated male pigs by using an miRNA microarray assay, thus providing a novel view of the role of miRNAs in castration-induced skeletal muscle growth and development.

2. Materials and methods

2.1. Animal experiments and sample collection

The experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee of

* Corresponding author. College of Animal Sciences, Zhejiang University, No. 388 Yuhangtang Road, Hangzhou 310058, China. Tel.: +86 571 88982089; fax: +86 571 88982089.
E-mail address: nyxu@zju.edu.cn (N. Xu).

http://dx.doi.org/10.1016/j.rvsc.2014.12.012
0034-5288/

Zhejiang University. A total of 14 male Large White pigs from seven pairs of full sibs were used in this experiment. On day 35, one pig from each pair was randomly selected to be castrated (CM) under anesthesia, and another one remained intact (IM). All animals were slaughtered at 210 days of age. The head, feet, and internal organs were removed, and individual carcass weight of both left and right sides were recorded. The left sides were physically dissected into bone, muscle, fat, and skin, which were also weighted individually. The LD muscle area was traced on an acetate film between the 10th and 11th ribs and subsequently determined by planimetry. Intramuscular fat content of the LD samples were determined with the Antaris meat analyzer (Thermo Electron Corporation, Waltham, MA, USA), working in the wavelength range of 780–2500 nm, based on the near infrared transmission (NIT) principle. The LD muscle samples were collected and frozen in liquid nitrogen, and then stored at –80 °C for subsequent experiments.

2.2. Measurement of serum testosterone levels

Blood samples were collected from male pigs at 35, 84, 147, and 210 days of age and were kept at room temperature for 2 h. Serum was collected by centrifugation (3000 × *g* for 15 min at 4 °C) and then stored at –80 °C until further use for the hormone assay. Serum testosterone levels were measured using a commercial radioimmunoassay kit (Beijing North Institute of Biotechnology, Beijing, China) according to the manufacturer's instructions.

2.3. Histological analysis

LD muscle samples were fixed in 10% neutral formalin solution, embedded in paraffin blocks, and sectioned to 6-μm thickness. The sections were stained with hematoxylin and eosin (H&E). Then, the slides were viewed and photographed using a digital camera mounted on an Olympus Microscope (Olympus BX51, Tokyo, Japan). The resulting photos were analyzed with ImageJ software (NIH, Bethesda, MD) as previously described (Wu et al., 2009).

2.4. miRNA microarray assay

Total RNA was extracted from LD muscle samples using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA integrity was measured using the Agilent 2100 BIOANALYZER® (Agilent Technologies, Waldbronn, Germany). RNA integrity number (RIN) values of the samples ranged from 8.2 to 9.8. RNA from three pairs of LD muscle samples from intact and castrated male pigs were extracted and pooled, respectively. MiRNA microarray analysis was performed by LC Sciences (Houston, TX, USA). The chip contained 236 known *Sus scrofa* miRNAs corresponding to miRNA transcripts listed in Sanger miRBase release 16.0 (http://www.sanger.ac.uk/Software/Rfam/mirna/) (Appendix: Supplementary Table S1). Each probe was spotted in duplicate, and control probes used as quality controls in chip production, sample labeling, and assay conditions were also included on the chip. The microarray assay was performed as described by Wang et al. (2013).

Data were analyzed by first subtracting the background and then normalizing the signals using a LOWESS filter (locally weighted regression) (Bolstad et al., 2003). The ratio of the two sets of detected signals (Log2 transformed, balanced) and *P*-values of the *t*-test were calculated (Pan, 2002). A transcript listed as detectable met the following conditions: signal intensity higher than 3 × (background standard deviation) and spot coefficient of variation (CV) less than 0.5. When repeating probes were present on an array, a transcript was listed as detectable only if the signals from at least 50% of the repeating probes were above the detection level (Li et al., 2012).

2.5. Stem-loop real-time RT-PCR

To validate the microarray results, stem-loop real-time RT-PCR was conducted to measure the expression levels of four selected miRNAs (Appendix: Supplementary Table S2) by using individual RNA samples that were pooled for use in the miRNA microarray assay. In brief, 1 μg total RNA was reverse transcribed using 0.5 μL M-MLV Reverse Transcriptase (200 U/μL) and 1 μL stem-loop RT primer (10 μM) in an Applied Biosystems 9700 Thermocycler with the following program: 42 °C for 60 min, followed by 70 °C for 15 min, and then held at 4 °C. Real-time PCR was performed using SYBR Green Master Mix (No. DRR041A, Takara, Japan) and the StepOne™ Software v2.0 (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer's instructions. Porcine miR-24 was used as an internal control, and all PCR reactions were run in triplicate. The comparative $2^{-\Delta\Delta Ct}$ method was used to determine differences in the expression levels (Livak and Schmittgen, 2001).

2.6. Target prediction and functional enrichment of differentially expressed miRNAs

TargetScan (Lewis et al., 2005) and miRanda (John et al., 2004) were used to predict the potential targets of differentially expressed miRNAs. The prediction results are a combination of these two queries. GO enrichment analysis was performed using the Gorilla tool (http://cbl-gorilla.cs.technion.ac.il/) (Eden et al., 2009; Joly-Tonetti et al., 2013). Probability values were corrected through the Benjamini and Hochberg's false discovery rate (FDR) method and considered significant if the corrected *P*-value was less than 0.05 (Joly-Tonetti et al., 2013). In addition, Cytoscape software (Cline et al., 2007) was used to construct the miRNA-mRNA network based on regulatory interactions between differentially expressed miRNAs and target GO genes.

2.7. Statistical analysis

Statistical analyses were performed using SPSS 13.0 software (SPSS, Chicago, IL, USA). A two-tailed Student's *t*-test was used to evaluate the statistical significance of differences between groups. The results were expressed as mean values with standard errors. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Effects of castration on serum testosterone levels and carcass characteristics in male pigs

The effect of castration on serum testosterone levels are shown in Fig. 1. Serum testosterone levels decreased significantly with castration. Total serum testosterone levels in intact male pigs were significantly higher than those of castrated male pigs at 35–210 days of age.

Castrated male pigs had lower body weight, lean meat weight, lean meat percentage and loin eye area (LEA) than intact pigs ($P < 0.05$; Table 1). Moreover, castration caused a significant reduction in the mean cross-sectional area (CSA) of LD muscle fibers in male pigs ($P < 0.05$; Fig. 2), along with decreased LEA and skeletal muscle mass in castrated male pigs. In contrast, castration resulted in a significant increase in carcass fat weight, fat percentage ($P < 0.01$) and intramuscular fat content ($P < 0.05$) in male pigs (Table 1).

3.2. miRNA microarray analysis

To examine castration-induced miRNA expression changes in skeletal muscle of male pigs, we compared differential miRNA expression

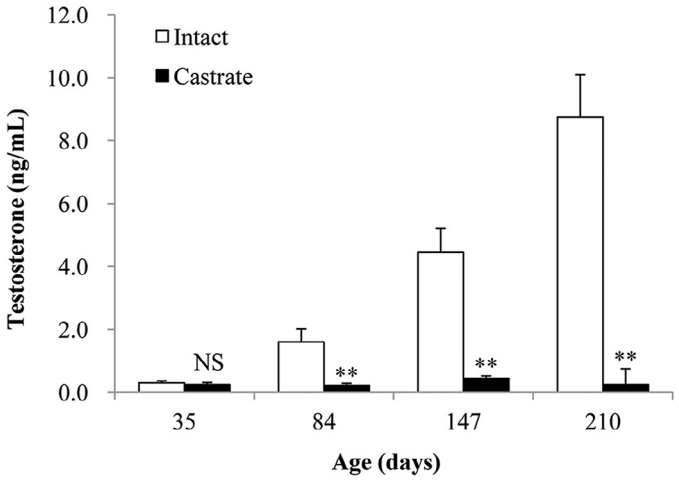

Fig. 1. Serum concentrations of testosterone in intact and castrated male pigs from 35 to 210 days of age. Values are shown as means ±standard error (SE), $n = 7$. $^{**}P < 0.01$, NS = not significant.

in LD muscle between intact and castrated male pigs by using a microarray assay. In total, 78 of 236 miRNA probes were detected. We defined miRNAs with signal values >500 and *P*-values <0.01 as the high signal group (Appendix: Supplementary Table S5). Among them, miRNAs with fold change in relative expression level ≥2 were considered differentially expressed miRNAs. Finally, seven miRNAs were identified as being differentially expressed between intact and castrated male pigs, including four up-regulated miRNAs (miR-1, miR-133a, miR-26a, and miR-133b) and three down-regulated miRNAs (miR-206, let-7c, and let-7a; Table 2).

Table 1
Effects of castration on carcass characteristics in male pigs.

Items	Intact	Castrate
Body weight (kg)	137.21 ± 7.51[a]	123.41 ± 4.35[b]
Lean weight (kg)	27.81 ± 1.39[A]	23.54 ± 0.92[B]
Lean percentage (%)	59.55 ± 1.49[A]	54.11 ± 1.00[B]
Fat weight (kg)	5.12 ± 1.00[A]	7.86 ± 0.85[B]
Fat percentage (%)	10.17 ± 1.54[A]	17.67 ± 1.12[B]
Longissimus muscle area (cm^2)	60.41 ± 1.35[a]	52.23 ± 0.86[b]
Intramuscular fat content (%)	1.88 ± 0.07[a]	2.43 ± 0.21[b]

Data are represented as mean ±standard error (SE), $n = 7$ for each group.
[a,b] Different superscripts indicate means within the rows that are significantly different ($P < 0.05$).
[A,B] Different superscripts indicate means within the rows that are significantly different ($P < 0.01$).

Table 2
Up-and down-regulated miRNAs in longissimus dorsi muscle of castrated male pigs.

Reporter name	Intact	Castrate	Regulation	Fold change	*P*-value[a]
ssc-miR-1	220	1963	Up	8.9	4.66E-15
ssc-miR-133a	1211	3659	Up	3.0	0.00E+00
ssc-miR-26a	190	501	Up	2.6	2.40E-06
ssc-miR-133b	1644	3877	Up	2.3	6.19E-13
ssc-miR-206	11369	1322	Down	8.6	0.00E+00
ssc-let-7c	804	128	Down	6.3	7.18E-09
ssc-let-7a	838	138	Down	6.1	9.44E-11

[a] The values are calculated using the mixture model approach as described by Pan (2002).

3.3. Validation of miRNA microarrays by stem-loop real-time RT-PCR

To validate the microarray results, the stem-loop real-time RT-PCR was performed to measure the expression levels of four selected differentially expressed miRNAs (miR-1, miR-133a-3p, miR-133b, and miR-206). The results showed that expression levels of miR-1, miR-133a-3p, and miR-133b were up-regulated, while the expression level of miR-206 was down-regulated in LD muscle of castrated male pigs (Fig. 3). The results of the stem-loop assay were consistent with microarray data.

3.4. Prediction and analysis of miRNA target genes

TargetScan and miRanda were used to predict potential target genes of seven differentially expressed miRNAs. A total of 1923 targets were successfully identified (Appendix: Supplementary Table S3). To define the biological functions of these target genes, GO analysis was carried out. Twenty-four GO terms involved in significantly enriched biological processes (corrected $P < 0.05$) were identified (Table 3 and Appendix: Supplementary Table S4), including G-protein coupled receptor signaling pathway, thioester biosynthetic process, biological regulation, response to extracellular stimulus, fatty acid transport, acyl-CoA biosynthetic process, organic substance transport, fatty acid metabolic process, actin-mediated cell contraction, actin-myosin filament sliding, monocarboxylic acid transport, and muscle filament sliding. The results indicate that these differentially expressed miRNAs and their

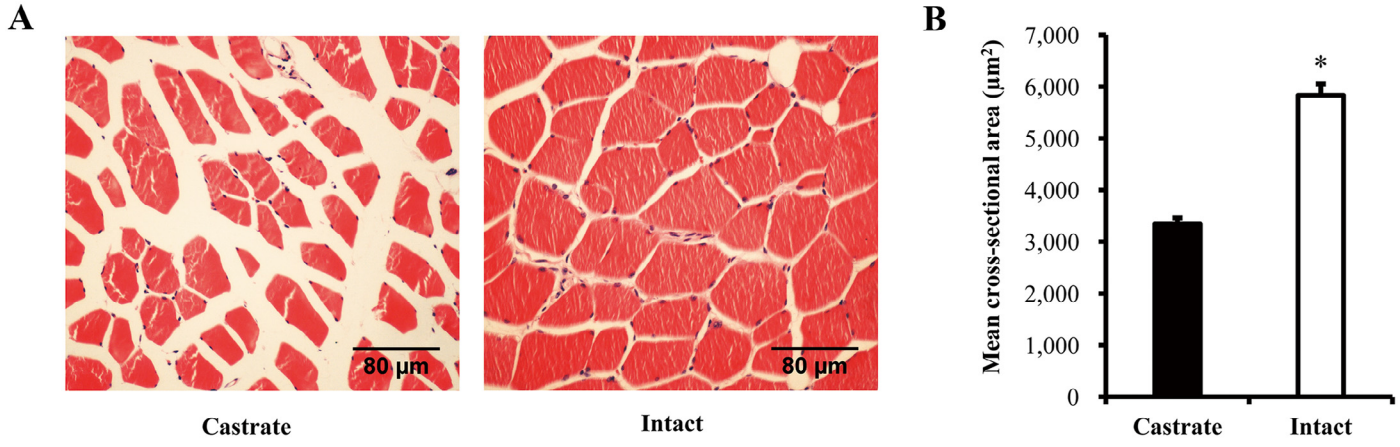

Fig. 2. Histological sections of LD muscle from intact and castrated male pigs. (A) H&E staining of longissimus dorsi muscle from intact and castrated male pigs (shown at ×200 magnifications). (B) The mean cross-sectional area of muscle fibers in intact and castrated male pigs. Cross-sectional area was measured using Image J software from three different animals per group (at least 50 fibers for each individual). $^{*}P < 0.05$.

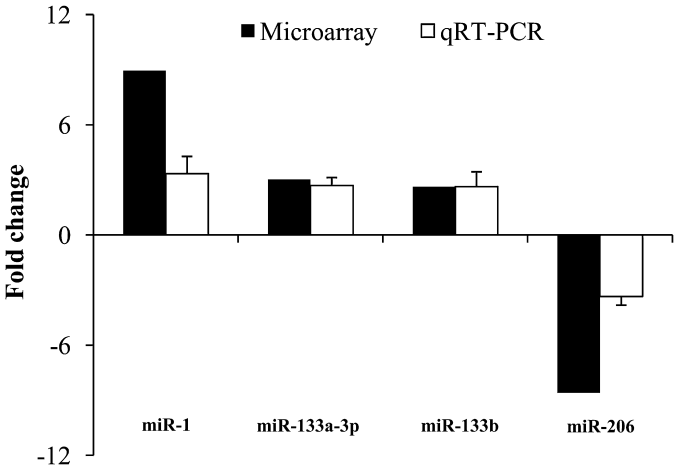

Fig. 3. Verification of miRNA microarray assay using real time PCR method. The fold change from the real-time PCR was determined using the $2^{-\Delta\Delta Ct}$ method. Data from real-time PCR are shown as the means ± standard error (SE) ($n = 3$). The black bars indicate the microarray results; whereas the white bars indicate the real-time PCR results.

target genes are involved in a wide range of regulatory functions in porcine skeletal muscle.

To gain insight into the interactions between miRNAs and their target genes, we carried out a miRNA–mRNA interaction network analysis (Fig. 4). The most highly connected regulators in the network were miR-133a and miR-206, each linked with 29 target genes. MiR-26a, a miRNA up-regulated with castration, regulates several important genes involved in skeletal muscle contractile function, i.e., *ACTC1* and *ACTN2*. Strikingly, let-7a and let-7c, two down-regulated miRNAs, had 22 common target genes. Among them, *ACSS2* and *ACSL6* were previously described to be involved in lipid synthesis in skeletal muscle. In addition, we identified some mRNAs as potential targets for four miRNAs, suggesting that a fine-tuned regulatory mechanism exists. We did not validate the predicted miRNA–target gene interactions in this study. This issue should be addressed in future studies.

4. Discussion

Castration is an effective tool to assess body changes under extreme testosterone deficiency in animal models. Previously, it was shown that castration of male rats increased body fat mass but reduced body lean mass (Jiao et al., 2009). Moreover, steers have lower skeletal muscle mass and higher lipid accumulation than intact bulls (Jeong et al., 2013). Similarly, in humans, men with testosterone deficiency show significant decreases in skeletal muscle mass and strength (Urban et al., 1995). Not surprisingly, our results also indicated that castration resulted in highly negative effects on skeletal muscle growth and positive effects on fat deposition in male pigs, which was consistent with other published reports (Gispert et al., 2010; Snockowski et al., 1981).

In the past few years, many groups have shown that miRNAs are involved in skeletal muscle growth and development (Dey et al., 2012; McDaneld et al., 2009; Zhao et al., 2012). To determine the roles of miRNAs involved in skeletal muscle development in castrated male pigs, we performed a miRNA microarray to investigate the effect of castration on miRNA expression profiles of LD muscle in male pigs. In the present study, seven miRNAs were identified as differentially expressed in LD muscle of intact and castrated male pigs. Among them, miR-1, miR-133a-3p, miR-133b, and miR-206 are skeletal muscle-specific miRNAs. A previous study found that miR-1 and miR-133a expression levels are reduced in a mouse model of skeletal muscle hypertrophy (McCarthy and Esser, 2007). Additionally, miR-206 expression levels are high in skeletal muscle of the mdx mouse, an animal model of muscular dystrophy (McCarthy et al., 2007). A recent study also revealed that miR-206 promotes skeletal muscle regeneration (Liu et al., 2012). These findings are consistent with our results, which show that miR-1, miR-133a-3p, and miR-133b are significantly increased, and miR-206 was significantly decreased in LD muscle of castrated male pigs. In general, muscle hypertrophy and atrophy are opposite conditions, and castration of male pigs results in loss of skeletal muscle mass (i.e., atrophy). Interestingly, in the present study, we found that two members of the let-7 miRNA family, let-7a and let-7c, were significantly down-regulated in LD muscle of castrated male pigs. Previous studies have shown that let-7 overexpression in mice results in decreased body fat mass (Frost and Olson, 2011). Let-7 inhibits adipogenic differentiation by down-regulating their target genes (Yan et al., 2013). We also identified many adipogenic marker genes such as *ME1*, *SCD*, *ACSS2*, and *ACSL6* that are associated with let-7a and let-7c levels (Appendix: Supplementary Table S3). Taken together, these findings provide strong evidence that decreased let-7 expression in castrated male pigs might correlate with higher fat tissue deposition. In addition, miR-26a was found to be highly abundant in skeletal muscle and up-regulated during muscle regeneration (Dey et al., 2012). This finding is inconsistent with our results, which show increased miR-26a levels in LD muscle of castrated male pigs. Although the underlying reasons remain unknown, a possible reason may result from the different experimental models of muscle disorders. Collectively, these muscle-specific or abundant miRNAs were considered differentially expressed in LD muscle between intact and castrated male pigs, suggesting they are regulated by sex hormones or play important roles in the regulation of skeletal muscle growth in castrated male pigs. However, thus far, few reports have addressed the effects of castration on miRNA expression in the skeletal muscle of pigs. We believe that our results provide a novel view of the role of miRNAs in the regulation of skeletal muscle growth and development.

In the present study, we used two different prediction tools, TargetScan and miRanda, to identify potential targets of the differentially expressed miRNAs. In total, 2540 target genes were predicted by TargetScan, 5137 target genes were obtained by miRanda, and 1923 target genes were predicted from both databases. Therefore, only the common target genes were annotated further. GO analysis revealed that 51 GO terms were enriched, such as actin filament organization, response to extracellular stimulus, actin-mediated cell contraction, fatty acid metabolic process, G-protein coupled receptor signaling pathway, actin-myosin filament sliding, muscle filament sliding, triglyceride biosynthetic process, and skeletal muscle thin filament assembly. Interestingly, many enriched terms correlated with muscle contractility. These results support the opinion that androgen influences muscle contractile function and the expression

Table 3
Top 10 enriched terms from targets of differentially expressed miRNAs.

GO term	Description	*P*-value	FDR *q*-value
GO:0007186	G-protein coupled receptor signaling pathway	5.26E-06	0.014
GO:0035384	Thioester biosynthetic process	2.03E-05	0.018
GO:0065007	Biological regulation	2.89E-05	0.019
GO:0009991	Response to extracellular stimulus	2.62E-05	0.020
GO:0015908	Fatty acid transport	3.86E-06	0.020
GO:0071616	Acyl-CoA biosynthetic process	2.03E-05	0.022
GO:0071702	Organic substance transport	1.84E-05	0.024
GO:0006631	Fatty acid metabolic process	1.59E-05	0.028
GO:0070252	Actin-mediated cell contraction	7.38E-05	0.030
GO:0033275	Actin-myosin filament sliding	7.38E-05	0.033

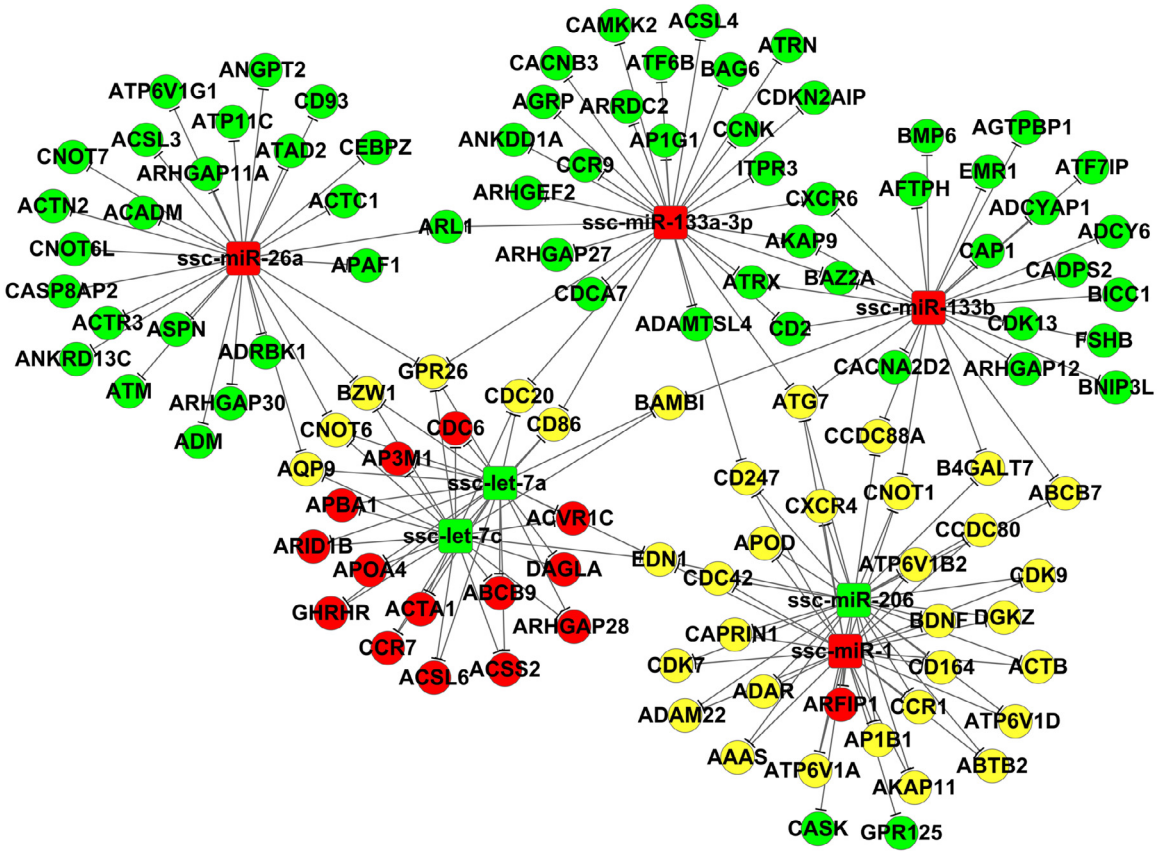

Fig. 4. The network of miRNA–target interactions between differentially expressed miRNAs and mRNAs associated with significant GO analysis. Rounded rectangle represent miRNAs (red – up-regulated, green – down-regulated); circles nodes represent genes (red – interaction with up-regulated miRNAs, green – interaction with down-regulated miRNAs, yellow – interaction with both up-regulated and down-regulated miRNAs); lines represent regulatory relations between miRNAs and genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of genes encoding muscle contractile proteins (MacLean et al., 2008; Yoshioka et al., 2007).

In conclusion, castration significantly reduces skeletal muscle mass and alters body composition in male pigs. More importantly, castration also causes significant changes in miRNA expression profiles in the longissimus dorsi muscle of male pigs. Our study identified muscle-specific and abundant miRNAs that may be directly involved in the development and function of skeletal muscle in castrated male pigs, providing a new clue for understanding the mechanisms underlying the effects of castration on porcine skeletal muscle growth and development.

Acknowledgements

This work is supported by grants from the National Natural Science Foundation of China (No. 31200921), Zhejiang Provincial Natural Science Foundation of China (No. LQ12C04003), and the Fundamental Research Funds for the Central Universities (KYZ201414).

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.rvsc.2014.12.012.

References

Bolstad, B.M., Irizarry, R.A., Astrandand, M., Speed, T.P., 2003. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics (Oxford, England) 19, 185–193.

Cline, M.S., Smoot, M., Cerami, E., Kuchinsky, A., Landys, N., Workman, C., et al., 2007. Integration of biological networks and gene expression data using Cytoscape. Nature Protocols 2, 2366–2382.

Dey, B.K., Gagan, J., Yan, Z., Dutta, A., 2012. miR-26a is required for skeletal muscle differentiation and regeneration in mice. Genes & Development 26, 2180–2191.

Eden, E., Navon, R., Steinfeld, I., Lipson, D., Yakhini, Z., 2009. Gorilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. BMC Bioinformatics 10, 48.

Frost, R.J., Olson, E.N., 2011. Control of glucose homeostasis and insulin sensitivity by the Let-7 family of microRNAs. Proceedings of the National Academy of Sciences of the United States of America 108, 21075–21080.

Gispert, M., Angels Oliver, M., Velarde, A., Suarez, P., Pérez, J., Font i Furnols, M., 2010. Carcass and meat quality characteristics of immunocastrated male, surgically castrated male, entire male and female pigs. Meat Science 85, 664–670.

Hou, X., Tang, Z., Liu, H., Wang, N., Ju, H., Li, K., 2012. Discovery of MicroRNAs associated with myogenesis by deep sequencing of serial developmental skeletal muscles in pigs. PLoS ONE 7, e52123.

Huang, Z., Chen, X., Yu, B., He, J., Chen, D., 2012. MicroRNA-27a promotes myoblast proliferation by targeting myostatin. Biochemical and Biophysical Research Communications 423, 265–269.

Jeong, J., Bong, J., Kim, G.D., Joo, S.T., Lee, H.J., Baik, M., 2013. Transcriptome changes favoring intramuscular fat deposition in the longissimus muscle following castration of bulls. Journal of Animal Science 91 (10), 4692–4704.

Jiao, Q., Pruznak, A.M., Huber, D., Vary, T.C., Lang, C.H., 2009. Castration differentially alters basal and leucine-stimulated tissue protein synthesis in skeletal muscle and adipose tissue. American Journal of Physiology. Endocrinology and Metabolism 297, E1222–E1232.

John, B., Enright, A.J., Aravin, A., Tuschl, T., Sander, C., Marks, D.S., 2004. Human MicroRNA targets. PLoS Biology 2, e363.

Joly-Tonetti, N., Vinuelas, J., Gandrillon, O., Lamartine, J., 2013. Differential miRNA expression profiles in proliferating or differentiated keratinocytes in response to gamma irradiation. BMC Genomics 14, 184.

Lewis, B.P., Burge, C.B., Bartel, D.P., 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120, 15–20.

Li, T., Chen, J., Qiu, S., Zhang, Y., Wang, P., Yang, L., et al., 2012. Deep sequencing and microarray hybridization identify conserved and species-specific

microRNAs during somatic embryogenesis in hybrid yellow poplar. PLoS ONE 7, e43451.
Liu, N., Williams, A.H., Maxeiner, J.M., Bezprozvannaya, S., Shelton, J.M., Richardson, J.A., et al., 2012. MicroRNA-206 promotes skeletal muscle regeneration and delays progression of Duchenne muscular dystrophy in mice. The Journal of Clinical Investigation 122, 2054–2065.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods (San Diego, Calif.) 25, 402–408.
MacLean, H.E., Chiu, W.S., Notini, A.J., Axell, A.M., Davey, R.A., McManus, J.F., et al., 2008. Impaired skeletal muscle development and function in male, but not female, genomic androgen receptor knockout mice. FASEB Journal 22, 2676–2689.
McCarthy, J.J., Esser, K.A., 2007. MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. Journal of Applied Physiology 102, 306–313.
McCarthy, J.J., Esser, K.A., Andrade, F.H., 2007. MicroRNA-206 is overexpressed in the diaphragm but not the hindlimb muscle of mdx mouse. American Journal of Physiology. Cell Physiology 293, C451–C457.
McDaneld, T.G., Smith, T.P., Doumit, M.E., Miles, J.R., Coutinho, L.L., Sonstegard, T.S., et al., 2009. MicroRNA transcriptome profiles during swine skeletal muscle development. BMC Genomics 10, 77.
Narayanan, R., Jiang, J., Gusev, Y., Jones, A., Kearbey, J.D., Miller, D.D., et al., 2010. MicroRNAs are mediators of androgen action in prostate and muscle. PLoS ONE 5, e13637.
Pan, W., 2002. A comparative review of statistical methods for discovering differentially expressed genes in replicated microarray experiments. Bioinformatics (Oxford, England) 18, 546–554.
Snockowski, M., Lundstrom, K., Dahlberg, E., Petersson, H., Edqvist, L.E., 1981. Androgen and glucocorticoid receptors in porcine skeletal muscle. Journal of Animal Science 53, 80–90.
Sutherland, M.A., Davis, B.L., Brooks, T.A., McGlone, J.J., 2010. Physiology and behavior of pigs before and after castration: effects of two topical anesthetics. Animal 4, 2071–2079.
Urban, R.J., Bodenburg, Y.H., Gilkison, C., Foxworth, J., Coggan, A.R., Wolfe, R.R., et al., 1995. Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. Americal Journal of Physiology 269, E820–E826.
von Deetzen, M.C., Schmeck, B., Gruber, A.D., Klopfleisch, R., 2013. Molecular quantification of canine specific microRNA species. Research in Veterinary Science 95 (2), 562–568.
Wang, H., Zheng, Y., Wang, G., Li, H., 2013. Identification of microRNA and bioinformatics target gene analysis in beef cattle intramuscular fat and subcutaneous fat. Molecular BioSystems 9, 2154–2162.
Wu, J.S., Liu, Y.H., Xu, N.Y., 2009. Histological characteristics of musculus longissimus dorsi and their correlation with restriction fragment length polymorphism of the myogenin gene in Jinghua × Pietrain F2 crossbred pigs. Meat Science 81, 108–115.
Wyce, A., Bai, Y., Nagpal, S., Thompson, C.C., 2010. Research resource: the androgen receptor modulates expression of genes with critical roles in muscle development and function. Molecular Endocrinology 24, 1665–1674.
Yan, X., Huang, Y., Zhao, J.X., Rogers, C.J., Zhu, M.J., Ford, S.P., et al., 2013. Maternal obesity downregulates microRNA let-7g expression, a possible mechanism for enhanced adipogenesis during ovine fetal skeletal muscle development. International Journal of Obesity (Lond) 37, 568–575.
Yoshioka, M., Boivin, A., Bolduc, C., St-Amand, J., 2007. Gender difference of androgen action on skeletal muscle transcritome. Journal of Molecular Endocrinology 39, 119–133.
Zhao, S., Zhang, J., Hou, X., Zan, L., Wang, N., Tang, Z., et al., 2012. OLFML3 expression is decreased during prenatal muscle development and regulated by microRNA-155 in pigs. International Journal of Biological Science 8, 459–469.