Gene 506 (2012) 211–216

Contents lists available at SciVerse ScienceDirect

Gene

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

Short Communication

Effects of polymorphisms in the porcine microRNA *miR-1* locus on muscle fiber type composition and miR-1 expression

Jae-Sang Hong [1], Su-Hyun Noh [1], Jun-Seong Lee [1], Jun-Mo Kim, Ki-Chang Hong, Young Sik Lee *

College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, South Korea

ARTICLE INFO

Article history:
Accepted 20 June 2012
Available online 27 June 2012

Keywords:
miR-1
Muscle fiber type composition
Pig
Polymorphism

ABSTRACT

MicroRNAs (miRNAs) are a group of evolutionarily conserved small noncoding RNAs with regulatory functions. Increasing evidence suggests that polymorphisms in miRNA genes are associated with phenotypic variation by affecting miRNA expression and/or function. Here, we identified two single nucleotide polymorphisms (SNPs) in the porcine *miR-1* locus, both of which were linked and located downstream from the stem–loop miRNA precursor sequence within the primary miR-1 region. An association study on muscle fiber characteristics and meat quality traits was performed with a total of 451 pigs representing three pig breeds (Berkshire, $n=153$; Landrace, $n=125$; Yorkshire, $n=173$). The *miR-1* SNPs were significantly associated with type I and type IIa muscle fibers in number and area compositions, respectively, but not with meat quality traits. Notably, these polymorphisms were also significantly associated with altered expression of the primary miR-1 transcript, ultimately leading to comparable changes in the levels of both precursor and mature miR-1. Furthermore, altered miR-1 levels were correlated with the variation in muscle fiber composition. Our data suggest that *miR-1* may be a candidate gene associated with muscle fiber type composition.

1. Introduction

Myogenesis is a complex process coordinated by networks of transcription factors that control muscle proliferation and differentiation (Buckingham, 2006). MicroRNAs (miRNAs) have emerged as a new level of gene regulators, playing crucial roles in a wide range of biological processes (Wienholds and Plasterk, 2005). For instance, increasing evidence has shown an important role for miRNAs in the regulation of muscle growth and differentiation by reinforcing transcriptional control (Nguyen and Frasch, 2006). Many miRNAs are expressed in a tissue-specific manner. Among them, a subset of conserved miRNAs including miR-1, miR-133, and miR-206 are expressed exclusively in skeletal and/or cardiac muscle and are referred to as myomiRs (Chen et al., 2006; Kim et al., 2006). Previous studies have shown that miR-1 and miR-206 enhance differentiation of myoblasts into myotubes, whereas miR-133 promotes myoblast proliferation by downregulating different target genes (Chen et al., 2006; Kim et al., 2006). Hence, changes in the function of myomiRs may significantly affect muscle phenotypes. Consistent with this idea, myomiRs have been implicated in muscle disorders such as muscle hypertrophy and muscular dystrophy (Eisenberg et al., 2007; McCarthy and Esser, 2007; McCarthy et al., 2007).

miRNAs are a class of small noncoding RNAs ~22 nucleotides in length that negatively regulate the expression of complementary target genes. The majority of miRNA genes are transcribed by RNA polymerase II as long, primary transcripts (pri-miRNAs) (Lee et al., 2004). A nuclear RNase III endonuclease called Drosha processes pri-miRNAs into approximately 70- to 90-nucleotide precursors (pre-miRNAs) with stem–loop structures (Lee et al., 2003). After export to the cytoplasm, pre-miRNAs are further processed by another RNase III endonuclease, Dicer, into mature miRNA duplexes (Hutvagner et al., 2001), of which one strand remains stably associated with the RNA-induced silencing complex. This complex recognizes and binds to the 3′ untranslated region (3′UTR) of complementary mRNAs, leading to translational repression or mRNA degradation (Chekulaeva and Filipowicz, 2009).

Genetic variations in miRNA genes can alter the biogenesis of miRNAs and/or their binding to target mRNAs (Georges et al., 2007; Slaby et al., 2012). Such polymorphisms may modulate the transcription of pri-miRNA transcripts and the stability or processing of pri- and pre-miRNAs, thereby leading to either an increase or decrease in mature miRNA levels. On the other hand, changes in the sequence of mature miRNAs can have an impact on their interaction with target mRNAs. Because miRNAs potentially control the expression of multiple targets, sequence variations in miRNA genes can have a functional effect and contribute to phenotypic variation via deregulation of target genes.

In this study, we identified two single nucleotide polymorphisms (SNPs) located in the pri-miR-1 region of the porcine *miR-1* locus and investigated their associations with muscle fiber characteristics and meat quality traits in pigs. We also examined the effect of the *miR-1* SNPs on the expression of miR-1. Collectively, our data suggest a potential role

Abbreviations: bp, base pair; HDAC, histone deacetylase; MEF2, myocyte enhancer factor-2; miRNA, microRNA; pre-miRNA, precursor microRNA; pri-miRNA, primary microRNA; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; UTR, untranslated region.

* Corresponding author. Tel.: +82 2 3290 3018; fax: +82 2 926 9746.
E-mail address: ys-lee@korea.ac.kr (Y.S. Lee).

[1] These authors contributed equally to this work.

0378-1119/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2012.06.050

for miR-1 in determining the fiber type composition of skeletal muscle in pigs.

2. Materials and methods

2.1. Animals and trait measurements

A total of 451 pigs consisting of Berkshire ($n=153$; 68 castrated males and 85 females), Landrace ($n=125$; 28 castrated males and 97 females), and Yorkshire ($n=173$; 53 castrated males and 120 females) breeds were included in this study. Each breed was raised under the same feeding conditions with commercial diet in different pens of the same farm, and slaughtered according to a standard protocol (Lee et al., 2012). Each animal was measured and recorded for muscle fiber characteristics and meat quality traits as described previously (Kim et al., 2009).

2.2. Polymorphism identification

Polymorphisms in the porcine *miR-1* locus (accession no. CU607084.3) on chromosome 17 (Cho et al., 2010) were detected using pooled genomic DNA isolated from the *longissimus dorsi* muscle of Berkshire, Landrace, and Yorkshire pigs, respectively. A 735-bp genomic fragment encompassing pre-miR-1 and its flanking sequences was amplified from the breed-specific DNA pools by polymerase chain reaction (PCR) with the miR-1-P1 primer pair (Table S1), as described previously (Lee et al., 2012). The resulting PCR products were purified using a PCR purification kit (Qiagen) and sequenced by Bionics Inc. Comparative sequence analysis for detecting polymorphisms was performed using SeqMan in DNASTAR software. The identified polymorphisms (g.123485C>T and g.123287G>A SNPs) were named according to the nomenclature guidelines for the description of sequence variations provided by the Human Genome Variation Society. The linkage between the two *miR-1* SNPs was assessed by DNA sequencing analysis of the PCR products amplified using the miR-1-P1 primers and the genomic DNA isolated from 78 randomly selected pigs from the Berkshire ($n=27$), Landrace ($n=25$), and Yorkshire ($n=26$) breeds.

2.3. Genotyping of miR-1 SNPs

The PCR-restriction fragment length polymorphism (PCR-RFLP) assay was used to genotype the *miR-1* SNPs in our experimental population of pigs, as described previously (Kim et al., 2010). The g.123287G>A SNP of the two *miR-1* SNPs was selected for genotyping due to its linkage. A PCR amplicon of 268 bp was amplified from genomic DNA isolated from the *longissimus dorsi* muscle using the miR-1-SNP primer pair (Table S1) and digested with the *Fau*I restriction enzyme. The digestion products were electrophoresed on an agarose gel and visualized with ethidium bromide.

2.4. Detection of mature miR-1

A quantitative reverse transcription-PCR approach, called miR-Q, was used to determine the levels of mature miR-1 (Sharbati-Tehrani et al., 2008). Total RNA was isolated from the frozen *longissimus dorsi* muscle of Berkshire pigs of the indicated genotypes using TRIzol (Invitrogen) according to the manufacturer's instructions and used for reverse transcription with M-MLV reverse transcriptase (Invitrogen) and the RT6-miR-1 primer (Table S1), according to the manufacturer's protocol. Quantitative PCR was performed on an iCycler iQ instrument (Bio-Rad) with SYBR Green SuperMix (Bio-Rad) with one short-miR-1-rev primer and two universal primers (Table S1). The levels of mature miR-1 were normalized to those of 5S rRNA, which was reverse transcribed with random hexamers and amplified by PCR with the 5S rRNA primers (Table S1).

2.5. Detection of pri- and pre-miR-1

Pri- and pre-miR-1 levels were determined using a quantitative reverse transcription-PCR-based method as described previously (Schmittgen et al., 2004). Briefly, total RNAs used for determining mature miR-1 levels were reverse transcribed with Thermoscript reverse transcriptase (Invitrogen) and the miR-1-R primer (Table S1), which anneals to the 3′ region of the pre-miR-1 sequence. Quantitative PCR was carried out with miR-1-pri-F and miR-1-R primers to detect pri-miR-1, and with miR-1-pri/pre-F and miR-1-R primers to detect both pri- and pre-miR-1 (Table S1). The relative levels of pri- and pre-miR-1 were calculated and normalized to the levels of 5S rRNA, which was reverse transcribed with random hexamers and amplified by PCR with the 5S rRNA primers.

2.6. Statistical analysis

Allele and genotype frequencies for the *miR-1* SNPs were determined in each breed and in the entire population of pigs. Associations between genotypes and measured traits were analyzed with the general linear model procedure using the statistical software package (SAS version 9.2; SAS Institute). The model was: $y_{ijklm}=\mu+G_i+B_j+S_k+P_l+GB_{ij}+e_{ijklm}$, where y_{ijklm} is the observation; μ is the general mean; G_i is the fixed effect of genotype i; B_j is the fixed effect of breed j; S_k is the fixed effect of sex k; P_l is the fixed effect of year-season l; GB_{ij} is the effect of the interaction between genotype and breed; e_{ijklm} is the random error. Significant differences in the levels of pri-, pre-, or mature miR-1 were determined with one-way analysis of variance. *P*-values of less than 0.05 were considered statistically significant.

3. Results

3.1. Identification and genotyping of polymorphisms in the miR-1 locus

In vertebrates, miR-1 is encoded by two loci from which the miR-1-1/133a-2 and miR-1-2/133a-1 pairs are transcribed as bicistronic primary transcripts on different chromosomes (Liu et al., 2007). We previously reported a porcine *miR-1/133a* cluster on chromosome 17 that corresponds to the *miR-1-1/133a-2* locus (Cho et al., 2010). Given the function of *miR-1* in promoting muscle differentiation (Chen et al., 2006), we searched for sequence variation in the porcine *miR-1* locus (accession no. CU607084.3). A 735-bp region encompassing pre-miR-1 and its flanking sequences was amplified from genomic DNA isolated from the *longissimus dorsi* muscle of Berkshire, Landrace, and Yorkshire pigs by PCR. Comparative sequence analysis of the resulting PCR products identified two SNPs (g.123485C>T and g.123287G>A) that are located 59 bp and 257 bp, respectively, downstream from the pre-miRNA sequence within the pri-miR-1 region (Fig. 1A).

The two *miR-1* SNPs were found to be linked as revealed by preliminary DNA sequencing analysis of PCR products containing both SNPs and thus are hereafter referred to as g.[123485C>T; 123287G>A]. The C allele of the g.123485C>T SNP was linked to the G allele of the g.123287G>A SNP, resulting in allele CG, and the T allele of the g.123485C>T SNP was linked to the A allele of the g.123287G>A SNP, leading to allele TA (Fig. 1B). We selected the g.123287G>A SNP for genotyping the *miR-1* SNPs with PCR-RFLP in a total of 451 pigs from Berkshire, Landrace, and Yorkshire breeds. A PCR product of 268 bp was amplified and digested with the *Fau*I restriction enzyme (Fig. 2). The genotype and allele frequencies are listed in Table 1. All pig breeds showed a higher frequency of allele CG than allele TA. The CCGG genotype was found in the highest frequency, and the CTGA genotype was more frequent than the TTAA genotype in all breeds.