Differential expression of microRNA-206 in skeletal muscle of female Piedmontese and Friesian cattle

Different expression of miRNA 206 in double-muscle Piedmontese female cattle

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Abstract

The double-muscle phenotype is a heritable condition in Piedmontese cattle traced to a point mutation in the myostatin gene. Analysis of SNP databases for humans demonstrates that mutations creating or destroying putative miRNA target sites are important effectors of phenotypic variation. Herein, we report the pattern of expression of miRNA 1 and 206 analysed in Piedmontese and Fresian cattle divided according to phenotype and sex. miR1 analysis showed no significant differences in expression between different muscle phenotypes in both genders and breed. Analysis of miR206 showed in female subjects a significant difference in expression between two breeds (four fold up-regulated in Piedmontese females than Friesian, $p<0.01$). Further studies are performing to investigate if changes in estrogens levels mediate response of skeletal muscle by miR-206 regulation of gene expression.

Keywords: muscle hypertrophy, Piedmontese cattle, miRNA206, miRNA1, female

The causes of muscle hypertrophy in Piedmontese cattle were traced to a point mutation in the myostatin gene (GA) amending the amino acid composition of the protein (cysteine-tyrosine) (Kambadur et al., 1997). This mutation alters the function of myostatin as a negative regulator of muscle growth thereby triggering muscle hypertrophy and hyperplasia (Berry et al., 2002). The muscular hypertrophy, or double-muscle phenotype, is a heritable condition in cattle that primarily result from an increase in number of muscle fibres (hyperplasia) rather than the enlargement of individual muscle fibres (hypertrophy), relative to normal cattle (Grobet et al., 1997). Piedmontese breed is one of the cattle breed in which this muscular hypertrophy and its effects have been analysed and it has been systematically selected for double muscling to the point of fixation in many herds (> 98% of homozygosity in Piedmonte region). MicroRNAs (miRNAs) are small non-coding RNA molecules, highly conserved, that regulate gene expression binding with imperfect complementarities the sequence of messenger RNA (mRNA). In the post-transcriptional gene regulation the mature miRNA not perfect matching with the non-coding region at 3 '(3'-UTR) mRNA target, inducing degradation. Through this mechanism, miRNAs are involved in a multitude of biological processes: differentiation, morphogenesis, development and cell regulation. miRNAs may be additive factors that explain the phenotypic variability in cattle selected for the myostatin gene mutation (De et al., 2008). It has been demonstrated that the GDF8 allele of Texel sheep, renowned for their exceptional meatiness, is characterized by a G to A transition in the 3' UTR that creates a target site for mir1 and mir206, microRNAs (miRNAs) that are highly expressed in skeletal muscle. This causes translational inhibition of the myostatin gene and hence contributes to the muscular hypertrophy of Texel sheep (Clop et al., 2006). Analysis of SNP databases for humans and mice demonstrates that mutations creating or destroying putative miRNA target sites are abundant and might be important effectors of phenotypic variation (Georges et al., 2006).

Herein, we report the pattern of expression of two miRNAs (206 and 1) analysed in Piedmontese and Fresian cattle divided according to phenotype and sex. Samples of loins were collected from 40 animals: 20 bred Piedmontese and 20 bred Friesian (from 16 to 23 months old), divided by gender and muscle phenotype through the use of grid assessment SEUROP (S = Super E = excellent, U = abundant, R = good; O = medium, P = low) based on visual estimate of muscle profiles of carcasses at slaughter. Samples were collected immediately after the slaughter and stored in RNA later ® (Ambion) until processing to prevent RNA degradation. RNA was extracted using TRIzol (Invitrogen); after RNA reverse transcription, Taq-Man miRNA Assays (Applied Biosystems) was performed for quantification of mature miR-1 and miR-206 expression levels. miR-16 was used to normalize the results.

The data were analysed by comparing: a) all samples Piemontese breed vs all samples of Friesian breed, b) samples of the two breed divided by gender, c) in Piedmontese breed, animals classified as S or E vs samples estimated as R or O. Through the use of bioinformatics software (UCSU browser; Rvista; Clustal), the degree of homology of miR1 and miR206 between humans and cattle was 100%.

The complete homology allowed us to use with confidence human Real Time TaqMan probes to evaluate the bovine miRNAs expression. miR1 analysis showed no significant differences in expression between different muscle phenotypes in both genders and breed. Analysis of miR206 showed in female subjects a significant difference in expression between two breeds (female Piedmontese: N = 10, mean ΔCt 4.9 ± 0.7; female Friesian: N = 10, mean ΔCt 2.8 ± 0.8, $p<0.01$). No significant differences in expression were reported between the two breeds (Piedmontese: N = 20, mean ΔCt 4.7 ± 0.7; Friesian: N = 20, mean ΔCt 4.3 ± 0.9) or within male gender (male Piedmontese: N = 10, mean ΔCt 4.6 ± 0.7, male Friesian: N = 10, mean ΔCt 4.3 ± 0.6).

The majority of the research on myomiRs has focused on the function of miR-1 and miR-133a in cardiac development and disease (Yang et al., 2007) or during skeletal muscle differentiation using an in vitro model system (Chen et al., 2006). miRNA-206 is a member of the muscle-specific miR-1 family of myomiRs. Though less studied, miR-206 is unique among the myomiR family specifically expressed in skeletal muscle, being absent or expressed at relatively low levels in other tissues. This suggests some intriguing possibilities on the study of miR-206 in skeletal muscle development and other potential functions in myogenesis and in adult skeletal muscle.

In our study, we did not observed differences in expression of miR-1 by comparing cattle breed, sex and muscle assessment. The mature form of miR-1 was found to be expressed only in the heart but not in the brain, kidney, liver, lung. The concept that this miRNA might have a role depending on its tissue-specific was further supported (Lagos-Quintana et al., 2002). On the other hand, within the development of muscle tissues, miR-1 does not appear as specific as miR-206 for skeletal muscle and this last microRNA appear to be more interesting to investigate different polymorphisms for this specific tissue. Even for miR-206 we did not observe any statistical difference within Piedmontese breed according to the different classification of carcass muscularity. However, miR-206 is four fold up-regulated in Piedmontese females than Friesian. We report the difference only in female animals, this observation lead us to hypothesize an influence of female hormonal milieu in the regulation of expression of miR-206 that may play a role in the determination of female phenotype. However, the relationship between estrogens and miR-206 is not clear, and an increased metastatic potential in human breast cancer cells associated with miR-206 has not been correlated with to ER status (Tavazoie et al., 2008). Further invstigation should now clarify if changes in estrogens levels may influence miR-206 regulation of gene expression in skeletal muscle

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper

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References

Berry, C., Thomas, M., Langley, B., Sharma, M., Kambadur, R., 2002. Single cysteine to tyrosine transition inactivates the growth inhibitory function of Piedmontese myostatin. American Journal of Physiology and Cell Physiol 283, C135-C141.

Chen, J.F., Mandel, E.M., Thomson, J.M., Wu, Q., Callis, T.E., Hammond, S.M., Conlon, F.L., Wang, D.Z., 2006. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nature Genetic 38, 228-233.

Clop, A., Marcq, F., Takeda, H., Pirottin, D., Tordoir, X., Bibe, B., Bouix, J., Caiment, F., Elsen, J.M., Eychenne, F., Larzul, C., Laville, E., Meish, F., Milenkovic, D., Tobin, J., Charlier, C., Georges, M., 2006. A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nature Genetic 38, 813-818.

De, S.C., Evans, B.S., Smith-Keune, C., Jerry, D.R., 2008. Molecular characterization, tissue expression and sequence variability of the barramundi (Lates calcarifer) myostatin gene. BMCGenomics 9, 82.

Georges, M., Clop, A., Marcq, F., Takeda, H., Pirottin, D., Hiard, S., Tordoir, X., Caiment, F., Meish, F., Bibe, B., Bouix, J., Elsen, J.M., Eychenne, F., Laville, E., Larzul, C., Milenkovic, D., Tobin, J., Charlier, A.C., 2006. Polymorphic microRNA-target interactions: a novel source of phenotypic variation. Cold Spring Harbour Symposium in Quantitative Biology 71, 343-350.

Grobet, L., Martin, L.J., Poncelet, D., Pirottin, D., Brouwers, B., Riquet, J., Schoeberlein, A., Dunner, S., Menissier, F., Massabanda, J., Fries, R., Hanset, R., Georges, M., 1997. A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nature Genetic. 17, 71-74.

Kambadur, R., Sharma, M., Smith, T.P., Bass, J.J., 1997. Mutations in myostatin (GDF8) in double-muscled Belgian Blue and Piedmontese cattle. Genome Research 7, 910-916.

Lagos-Quintana, M., Rauhut, R., Yalcin, A., Meyer, J., Lendeckel, W., Tuschl, T., 2002. Identification of tissue-specific microRNAs from mouse. Current Biology 12, 735-739.

Tavazoie, S.F., Alarcon, C., Oskarsson, T., Padua, D., Wang, Q., Bos, P.D., Gerald, W.L., Massague, J., 2008. Endogenous human microRNAs that suppress breast cancer metastasis. Nature 451, 147-152.

Yang, B., Lin, H., Xiao, J., Lu, Y., Luo, X., Li, B., Zhang, Y., Xu, C., Bai, Y., Wang, H., Chen, G., Wang, Z., 2007. The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. Nature Medicine 13, 486-491.

Table 1

a)

miRNA	Piedmontese	Friesian
miR-1	3.9 ±0.5	4.1 ±0.5
miR-206	4.7 ±0.7	4.4 ±0.9

b)

	miRNA	Piedmontese	Friesian
Male	miR-1	4.0 ±1.1	4.2 ±1.1
	miR-206	4.3 ±0.9	3.7 ±0.5
Female	miR-1	3.9 ±0.4	3.7 ±0.7
	miR-206	4.9 ±0.4*	2.8 ±0.8*

c)

miRNA	Piemontese phenotypes S.E.U.R.O.P.	
	S/E	U/R/O
miR-1	3.8 ±0.5	4.1 ±0.3
miR-206	4.7 ±0.02	4.7 ±0.9

d)

CLUSTAL 2.0.12 multiple sequence alignment

bta-miR-1
UGGAAUGUAAAGAAGUAUGUAU
hsa-miR-1
UGGAAUGUAAAGAAGUAUGUAU

bta-mir-206
UGGAAUGUAAGGAAGUGUGUGG
hsa-mir-206
UGGAAUGUAAGGAAGUGUGUGG

Tab. 1 a) miR-1 expression in Piedmontese and Friesan animals; b) miR-206 expression in Piedmontese and Friesan animals; c) miR-1 and miR-206 expression within piedmontese animals divided according to the grid assessment SEUROP; d) sequence of miR1 and miR206 in cattle vs humans