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Research paper

MiR-183 promotes preadipocyte differentiation by suppressing *Smad4* in goats

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ABSTRACT

As a well-conserved microRNA, miR-183 is ubiquitously expressed in many tissues and cells including backfat and the 3T3-L1 adipocytes; however, the mechanisms regulating miR-183 in adipogenesis remain poorly understood. Here, we explored the expression pattern and role of miR-183 in adipogenesis using hircine preadipocytes. The results showed that miR-183 was up-regulated during preadipocyte differentiation, and overexpression of miR-183 enhanced lipid accumulation and dramatically increased the mRNA expression of the adipogenic genes *PPARγ*, *C/EBPα*, *SREBP-1c*, *FAS*, and *ACC*. Using bioinformatics tools, we predicted *Smad4* to be a target of miR-183. This was subsequently validated with a luciferase reporter assay. Overexpression of miR-183 suppressed the mRNA and protein levels of *Smad4* significantly, whereas inhibiting miR-183 had the opposite effect. However, inhibition of *Smad4* greatly accelerated lipid deposition and increased the expression of adipogenic genes which consists with the results of miR-183 overexpression. In conclusion, these results indicate that miR-183 promotes hircine preadipocyte differentiation by targeting *Smad4*.

1. Introduction

Adipogenesis, a complex process in which fibroblast-like preadipocytes differentiate into lipid-laden and insulin-responsive mature adipocytes (Lefterova and Lazar, 2009), requires sequential activation of numerous transcription factors and noncoding RNAs. Among these, peroxisome proliferator-activated receptor γ (*PPARγ*) (Tontonoz et al., 1994) and CCAAT/enhancer binding protein α (*C/EBPα*) (Yeh et al., 1995; Rosen et al., 2002), known as adipogenic master genes, promote adipogenesis by activating genes such as fatty acid-binding protein 4, fatty acid synthase, and lipoprotein lipase. Reversely, the differentiation of adipocytes is inhibited by *Smad4* via the transforming growth factor-β (TGF-β) signaling pathway (Ignotz and Massagué, 1985; Choy et al., 2000). In addition, microRNAs, a class of short noncoding RNAs that are generally regarded to inhibit gene expression by binding to the 3′-UTR of target mRNAs (Lagos-Quintana et al., 2001; Bagga et al., 2005), have been confirmed to regulate lipid metabolism in animals. Studies on human preadipocytes have demonstrated that miR-27 impairs human adipocyte differentiation by targeting *PPARγ* (Kim et al., 2010), while miR-103 enhances adipogenesis by inhibiting *PDK1* (Wilfred et al., 2007). In the mouse, the role of several miRNAs has been studied during 3T3-L1 adipocyte differentiation. MiR-210 and the miR-17-92 cluster promote the differentiation of preadipocytes (Wang et al., 2008; Liang et al., 2013), while miR-302a represses mouse adipogenesis (Jeong et al., 2014). Furthermore, miR-143 (Esau et al., 2004) and miR-375 (Ling et al., 2011) promote adipogenesis, whereas miR-124 (Qadir et al., 2014) and miR-199a-5p (Alexander et al., 2013) have the opposite effect.

MiR-183 is a well-conserved microRNA across many species from invertebrates to humans. Many studies have unveiled the functions of miR-183 in neurosensory development (Pierce et al., 2008), tumor cell migration (Lowery et al., 2010), non-small cell lung cancer development (Zhang et al., 2015), and inner ear development (Sacheli et al., 2009). In pigs, the expression of miR-183 is higher in the backfat of Meishan pigs (Chinese indigenous fatty pig) than in Large White pigs (lean breed of pig) (Chen et al., 2012). During 3T3-L1 pre-adipocyte differentiation, miR-183 levels are increased, which subsequently promotes adipogenesis by targeting low-density lipoprotein receptor-

Abbreviations: PPARγ, peroxisome proliferator-activated receptor γ; *C/EBPα*, CCAAT/enhancer binding protein α; *FAS*, fatty acid synthase; TGFβ, transforming growth factor β; *LRP6*, low density lipoprotein receptor related protein 6; *SREBP-1c*, sterol regulatory element binding protein 1c; *ACC*, acetyl-CoA carboxylase; *C/EBPβ*, CCAAT/enhancer binding protein β; *C/EBPδ*, CCAAT/enhancer binding protein δ; *PDK1*, phosphoinositide-dependent kinase-1

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Table 1
Primers used in this study.

Gene	Primer	Primer sequence (5′-3′)	Tm/°C	Product size/bp
PPARγ	PPAR-F	GTGTCACTCCTGAACGAAAT	56.4	156
	PPAR-R	GGAAATGCTGGAGAAGTCAA		
C/EBPα	CEBP-F	CCGTGGACAAGAACAGCAA	58.4	141
	CEBP-R	GGCGGTCATTGTCACTGG		
SREBP1	SREBP-F	CTGCTGACCGACATAGAAGACAT	56.4	88
	SREBP-R	GTAGGGCGGGTCAAACAGG		
ACC	ACC-F	CCGTCTGTGATGACTTTGA	56.4	191
	ACC-R	CTTTCTGGGTTGGGTGAG		
FAS	FAS-F	GAAGGGTGTTGACCTCGTCC	58.4	126
	FAS-R	AGAGGGTGGTTGTTGGAAAG		
SMAD4	SMAD4-F	GACAGCAGCAGAATGGAT	58.5	225
	SMAD4-R	CAGGAGCAGGATGATTAGAA		
ACTB	ACTB-F	CCTGCGGCATTCACGAAACTAC	59.7	87
	ACTB-R	ACAGCACCGTGTTGGCGTAGAG		
miR-183	183-F	TATGGCACTGGTAGAATTCACT	55.9	–
U6	U6-F	CAAGGATGACACGCAAATTCG	55.9	–
3′-UTR	UTR-F	CCGCTCGAGGTGTGAGTTGTTA CTGTTGA	58.5	185
	UTR-R	AAATATGCGGCCGCTCGCAAGA CAATCTGAGTT		
3′-mut	mut-F	AAATATT ACTATGGGTGGGT	55	–
	mut-R	CGGAGTAAAGTATGTTCAAC		

related protein 6 (*LRP6*) (Kajimoto et al., 2006; Chen et al., 2014). Given the many-to-many relationships between miRNAs and their targets (Sarver et al., 2010), the various mechanisms employed by miR-183 to regulate preadipocyte differentiation warrants investigation.

As an economically important animal, domestic goats primarily serve as a major source of meat. Adipose tissue is directly associated with the yield and quality of meat. Previous studies have revealed that miR-183 plays a positive role in mouse adipogenesis (Kajimoto et al., 2006; Chen et al., 2014); however, its function in goat adipocyte development remains unclear. In this study, we studied the roles and potential mechanisms of miR-183 in goat adipogenesis. Our results revealed that overexpression of miR-183 accelerated the expression of adipogenic marker genes and lipid accumulation during hircine preadipocyte differentiation, while repressing miR-183 attenuated lipid accumulation and adipogenic gene expression. We also validated *Smad4* as a target gene of miR-183. Decreasing *Smad4* promoted adipogenesis, yielding the same effect as the overexpression of miR-183. Collectively, these results unveiled that miR-183 plays a positive role in hircine preadipocyte differentiation via the suppression of *Smad4*, which clarifies the roles of miRNA in goat adipogenesis.

2. Materials and methods

2.1. Cell isolation

All of the experimental procedures for this experiment were

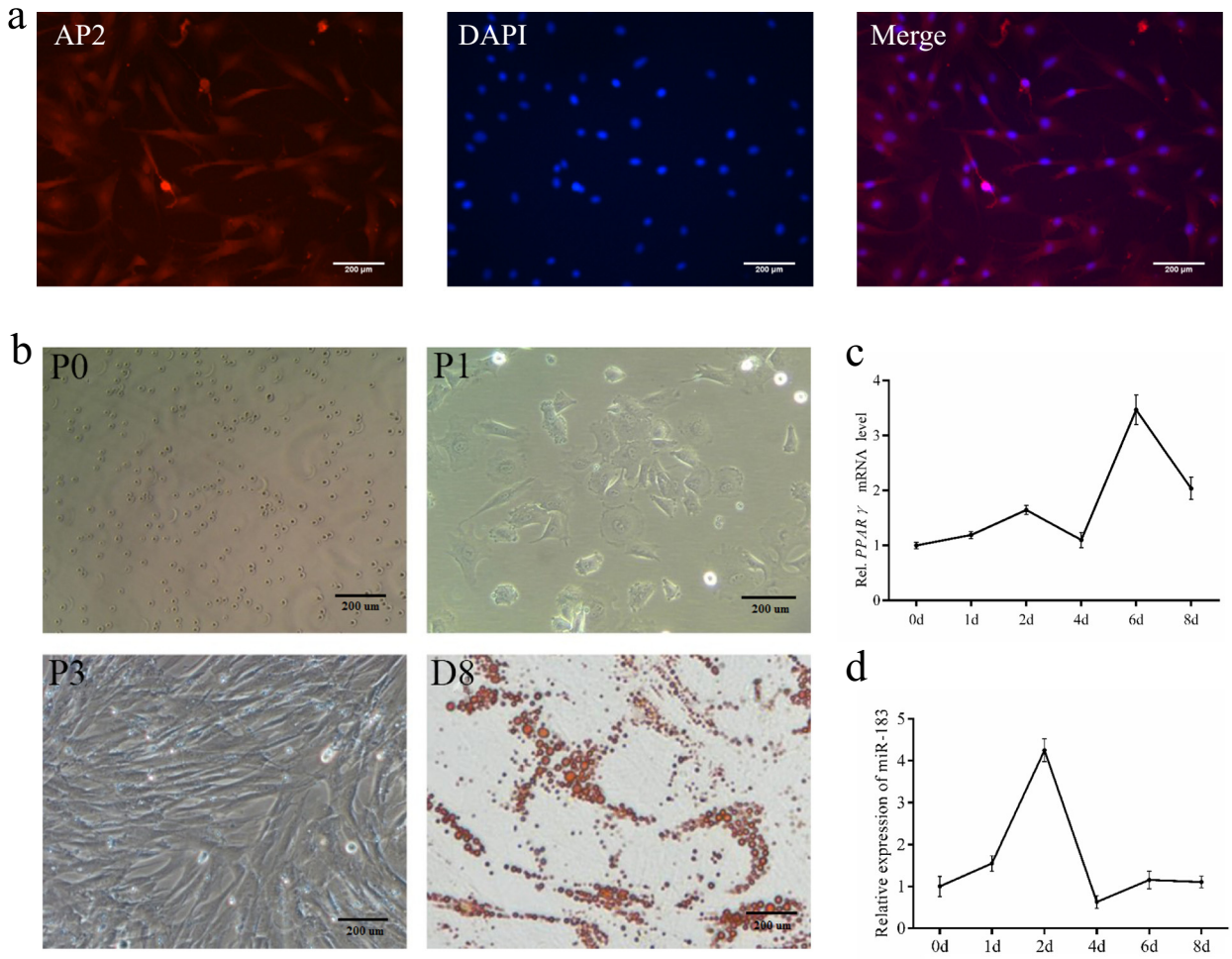

Fig. 1. Expression profile of miR-183 during hircine preadipocytes differentiation. The immunofluorescent staining of AP2 in preadipocytes (a), cell morphology change (b), *PPARγ* mRNA expression (c), and the temporal expression pattern of miR-183 (d) during adipogenesis. P0, newly isolated hircine preadipocytes. Preadipocytes cultured for 24 h (P1) and 3 d (P3) in growth medium. D8, adipocytes staining with Oil Red O.