Biochemical and Biophysical Research Communications 463 (2015) 624–631

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Identification of miR-2400 gene as a novel regulator in skeletal muscle satellite cells proliferation by targeting MYOG gene

Wei Wei Zhang [a, b], Hui Li Tong [a], Xiao Feng Sun [a], Qian Hu [a], Yu Yang [a], Shu Feng Li [a], Yun Qin Yan [a, *], Guang Peng Li [c]

[a] The Laboratory of Cell and Development, Northeast Agricultural University, Harbin, Heilongjiang 150030, China
[b] College of Life Sciences and Agriculture & Forestry, Qiqihar University, Qiqihar, Heilongjiang 161006, China
[c] The Key Laboratory of Mammal Reproductive Biology and Biotechnology Ministry of Education, Inner Mongolia University, Hohhot 010021, China

ARTICLE INFO

Article history:
Received 19 May 2015
Accepted 29 May 2015
Available online 3 June 2015

Keywords:
miR-2400
Skeletal muscle-derived satellite cells
Proliferation
Myogenin
CRISPRi

ABSTRACT

MicroRNAs play critical roles in skeletal muscle development as well as in regulation of muscle cell proliferation and differentiation. Previous study in our laboratory showed that the expression level of miR-2400, a novel and unique miRNA from bovine, had significantly changed in skeletal muscle-derived satellite cells (MDSCs) during differentiation, however, the function and expression pattern for miR-2400 in MDSCs has not been fully understood. In this report, we firstly identified that the expression levels of miR-2400 were down-regulated during MDSCs differentiation by stem-loop RT-PCR. Over-expression and inhibition studies demonstrated that miR-2400 promoted MDSCs proliferation by EdU (5-ethynyl-2′ deoxyuridine) incorporation assay and immunofluorescence staining of Proliferating cell nuclear antigen (PCNA). Luciferase reporter assays showed that miR-2400 directly targeted the 3′ untranslated regions (UTRs) of myogenin (MYOG) mRNA. These data suggested that miR-2400 could promote MDSCs proliferation through targeting MYOG. Furthermore, we found that miR-2400, which was located within the eighth intron of the Wolf-Hirschhorn syndrome candidate 1-like 1 (WHSC1L1) gene, was down-regulated in MDSCs in a direct correlation with the WHSC1L1 transcript by Clustered regularly interspaced palindromic repeats interference (CRISPRi). In addition, these observations not only provided supporting evidence for the codependent expression of intronic miRNAs and their host genes in vitro, but also gave insight into the role of miR-2400 in MDSCs proliferation.

1. Introduction

MicroRNAs are a family of non-coding small RNAs, approximately 22 nucleotides in length, that mainly repress the translation and accelerate the decay of mRNAs through pairing of their seed sequences with the 3′ UTRs of target genes [1] and may regulate up to 30% of gene expressions [2]. MicroRNAs have been shown to play critical roles in skeletal muscle development and in the regulation of muscle cell proliferation and differentiation [3,4]. Wang et al. [5] and Motohashi et al. [6] had Identified the miRNAs which involved in bovine skeletal muscle satellite cell myogenic differentiation. Some studies indicate that miRNA-1 and miR-206 promote the differentiation of myoblasts, whereas miR-133 promotes proliferation [7–9]; miR-27 modulates the entry of cells into the myogenic differentiation program [10]; miR-214 promotes myogenic differentiation by facilitating the exit of myoblasts from the cell cycle [11]; and miR-181 promotes myogenesis [12]. MicroRNA-139-5p regulates C2C12 cell myogenesis through blocking Wnt/β-catenin signaling pathway [13]. MicroRNA-128 targets myostatin at coding domain sequence to regulate myoblasts in skeletal muscle development [14]. Although an increasing body of evidence shows that miRNAs play important roles in the regulation of skeletal muscle proliferation, the functional role and molecular mechanism are not fully understood.

MicroRNA-2400, one of miRNA, is a novel and unique miRNA from bovine according to Glazov et al. [15]. It located at BTA27 and within introns 8 of WHSC1L1 gene. It has showed that the expression level of miR-2400 had significantly changed in MDSCs during differentiation by Deep Sequencing, however, the function and expression pattern for miR-2400 in myoblast proliferation has

* Corresponding author. The Laboratory of Cell and Development, Northeast Agricultural University, Mucai Street 59, Xiangfang District, Harbin, 150030 Heilongjiang Province, China.
E-mail address: yanyunqin@sohu.com (Y.Q. Yan).

http://dx.doi.org/10.1016/j.bbrc.2015.05.112
0006-291X/

not been reported. In the present study, we investigated the role of miR-2400 in bovine MDSCs proliferation and demonstrated that miR-2400 targeted the 3′UTR of MYOG transcript and down-regulate MYOG expression, thereby promoted MDSCs proliferation. CRISPRi and dual-luciferase reporter assay showed that miR-2400 was processed from the host gene WHSC1L1 and co-expressed with its host gene.

2. Materials and methods

2.1. Cell culture

Sample collection from animals was approved by the Animal Care Commission of the Northeast Agricultural University and Heilongjiang, P.R. China. Cells were isolated from hindlimb muscles of newborn Chinese Simmental calves according to a method described previously [16]. MDSCs were maintained in growth medium (GM) (containing DMEM (Gibco Invitrogen, Carlsbad, CA, USA), 20% FBS, 100 U/ml penicillin and 100 μg/mL streptomycin (Invitrogen)) at 37 °C in the 5% CO_2 humidified atmosphere. The cells were transfected with plasmid DNA, or miR-2400 inhibitor (RiboBio, Guangzhou, China) by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction.

2.2. Plasmid construction

For bta-miR-2400 over-expression, the pre-miR-2400 was PCR-amplified from bovine genomic DNA and cloned into pcDNA3.1 (+) plasmid (Promega, Madison, WI, USA) to generate pcDNA3.1 (+)-miR-2400. For MYOG 3′UTR reporter assay, the entire 3′ UTR of bovine MYOG was PCR-amplified from bovine genomic DNA and cloned into psiCHECK-2 dual-luciferase reporter plasmid (Promega) to generate psiCHECK-2-MYOG-wt. The mutant bovine MYOG 3′UTR reporter, designated as psiCHECK-2-MYOG-mu, was created by mutating the seed region of the predicted bta-miR-2400 site (TGTGCTGA to ACACGTGA) by nested PCR. For bta-miR-2400 promoter reporter assay, the intron 8 of WHSC1L1 gene which contained the pre-miR-2400 was PCR-amplified from bovine genomic DNA and cloned into pGL3-Basic plasmid (Promega) to generate pGL3-intro-miR-2400. The primers used in plasmid construction were shown in Table 1.

2.3. Cell proliferation assay

MDSCs were seeded and transfected with pcDNA3.1 (+), pcDNA3.1 (+)-miR-2400, miR-2400 inhibitor Negative Control (miR-2400-NC) or miR-2400 inhibitor (miR-2400-I). Then, the cells were maintained in growth medium for 24 h. For EdU incorporation assay, proliferating cells were determined by using the Cell-Light™ EdU Apollo®488 In Vitro Imaging Kit (RiboBio) according to the manufacturer's protocol. For general immunofluorescence (IF) staining assay of PCNA, The cells were fixed with 4% paraformaldehyde (PFA) for 20 min and treated with 1% Triton X-100 in PBS (PBST) for 10 min at room temperature, then blocked with 5% BSA in PBST for 1 h. After incubation with primary antibodies against PCNA for 1 h at 37 °C, the cells were incubated against FITC-conjugated second antibodies. The slides were co-stained with 4,6-diamidino-2-phenylindole (DAPI) to visualize the nuclei. The relative area of positive staining was evaluated using Image J software. For quantification analysis, each data point represents the positive fluorescence area calculated from a minimum of five randomly chosen fields from three individual experiments.

2.4. Luciferase reporter assay

To explore the mechanisms of miR-2400-induced cell proliferation, we searched for the target genes of miR-2400 by luciferase reporter assay. HeLa cells (2.0×10^4 cells per well) were plated in a 24-well plate (**Corning, NY, USA**) 24 h before transfection. Cells were co-transfected with 0.5 μg of either the psiCHECK-2-MYOG-wt, psiCHECK-2-MYOG-mu or empty vector psiCHECK-2, and pcDNA3.1(+), pcDNA3.1(+)-miR-2400, miR-2400-NC or miR-2400-I. After transfection of 48 h, cells were lysed in Passive Lysis Buffer (Promega) and activities of Firefly and Renilla luciferase were measured with a GloMax 20/20 Luminometer (Promega) using the Dual-Luciferase Reporter Assay System according to the manufacturer's protocols.

2.5. Real-time PCR of MYOG and miR-2400

Total RNA was extracted from the transfected cells using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. The concentrations of total RNA were determined spectrophotometrically using a NanoDrop 2000C Spectrophotometer (Thermo, USA). A first-strand cDNA was prepared from 1 μg of total RNA from each sample using a TransScript® One-Step gDNA Removal and cDNA Synthesis Super Mix (Beijing TransGen Biotech Co., Ltd) according to the protocols of the manufacturer. The cDNA was then used for real-time PCR of miR-2400 and MYOG. The bovine 18S ribosomal RNA (18S) gene and β-actin were used as endogenous control respectively. Real-time quantitative PCR was performed using an ABI7300 Real Time Detection System. The PCR mix included 100 ng cDNA for each miRNA, 0.4 μM forward and reverse primers, and 10 μL 2 × SYBR Real-time PCR Premix. The cycle conditions were as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 30 s. Relative expression level was analyzed using the comparative Ct method [17]. All reactions were performed in triplicate, the quantification of miR-2400 and MYOG relative to endogenous control gene was calculated by the formula: $N = 2^{-\Delta\Delta Ct}$. The primers for miRNAs and the control gene were shown in Table 2.

2.6. Western blot analysis

Protein samples were prepared from MDSCs. Briefly, the cells were rinsed twice with ice cold PBS and put in lysis buffer and then incubated for 30 min on ice. The cell lysates were centrifuged (12,000 rpm) at 4 °C for 15 min. The resultant samples were resolved by electrophoresis on a 12% SDS-polyacrylamide gel and then transferred to a PVDF membrane (Millipore Corporation, USA). The membrane was incubated with a primary antibody (anti-MYOG (5FD), anti-GAPDH (6C5, Santa Cruz, USA)) followed by the addition

Table 1
Primers used in plasmid construction.

Gene	Forward primer	Reverse primer
pre-miR-2400	CGGGGTACCAACCCTGGGGCTGCTTGT	CCGGAATTCCATGGTCCCG TTTTCTGG
Myog-3UTR	CCGCTCGAGatctgaccaaggtctctgtgctgaagttgc	ATAAGAATGCGGCCGCCTAGCACCCAGTCTTTATTT
T-Myog-3UTR	CCGCTCGAGatctgaccaaggtctcacacgtgaagttgc	ATAAGAATGCGGCCGCCTAGCACCCAGTCTTTATTT
miR-2400-intron	TGAAGTTGCGACTTTGCC	AATCTGGACATGGTCCCGTTT

Table 2
Primers used in semi-quantitative RT-PCR.

Gene	Forward primer	Reverse primer
miR-2400	ACACTCCAGCTGGGCCAGCACAGGCAGCTCGGA	CTCAACTGGTGTCGTGGA
18S	GGACATCTAAGGGCATCACAG	AATTCCGATAACGAACGAGACT
MYOG	GACTCAAGAAGGTGAATGAAGCC	TATTATAGTGCGCTGCCCCAC
β-actin	GACCTCTACGCCAACACG	GCAGCTAACAGTCCGCCTA
miR-2400-RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAGTCCG	

of a secondary antibody (HRP-labeled goat anti-mouse or rabbit IgG (Santa Cruz, USA)). The proteins were visualized by Super ECL Plus detection kit (Applygen Technologies Inc., Beijing, China) according to the manufacture's instruction. The membranes were exposed to Kodak X-Omat film for various times.

2.7. CRISPRi interference WHSC1L1 transcription

To demonstrate the relationship between the expression of miR-2400 and WHSC1L1, Four sgRNAs targeting different sites of WHSC1L1 (ID: 513054) promoter were designed following Matthew H L et al. [18,19]. Each sgRNA contaied 20 nt target sequence and 4 nt Bbs I restriction site at the 5′ ends. N1 : CACCGGCCTGGCACGTGATGGGCG; N2 : CACCGGCTGAAGATTTGGC GG; N3: CACCGCGGCGGCGGCGGGGGCGCC; N4: CACCGGCCAG-GACGGAGGCGG. These oligonucleotides were synthesized, annealed and ligated into the Bbs I sites under hU6 Promoter of pSPgRNA expression vector (Addgene, UK). The ligation products were transformed into *E. coli* DH5α; positive transformed clones were selected, and sequenced. For functional assay, MDSCs were plated in a 6-well plate 24 h before transfection. Cells were co-transfected with 2 μg of the dCas9 expression plasmid and 2 μg of the sgRNA expression plasmid. Total RNA was extracted after transfected 48 h, and real-time RT-PCR was used to detect the expression of WHSC1L1 and miR-2400.

2.8. Statistical analysis

For all data expressed as mean ± SEM were compared using ANOVA followed by Tukey's tests (SPSS Inc., USA). Differences were regarded as significant at a level of $P < 0.05$.

3. Results

3.1. MiR-2400 was downregulated in MDSCs during differentiation

Previous study in our laboratory showed that the expression level of miR-2400 was down-regulated in MDSCs during differentiation. To further investigate whether miR-2400 is involved in proliferation, we firstly determined the expression levels of miR-2400 in MDSCs during differentiation by stem-loop RT-PCR. The results showed that the expression levels of miR-2400 were down-regulated during MDSCs differentiation (Fig. 1).

3.2. MiR-2400 promoted MDSCs proliferation

We demonstrated that the expression level of miR-2400 in pcDNA3.1 (+)-miR-2400 transfected MDSCs was higher than that in control by qRT-PCR(Fig. 2a). To verify whether miR-2400 promote MDSCs proliferation, we performed an EdU incorporation assay and IF staining of PCNA. After transfected with pcDNA3.1 (+), pcDNA3.1 (+)-miR-2400, miR-2400-NC or miR-2400-I, EdU incorporation experiments and IF staining of PCNA were performed to assess its proliferation. As shown in Fig. 2b, compared with control, the cell proliferation rate was significantly increased by 33.3% in MDSCs

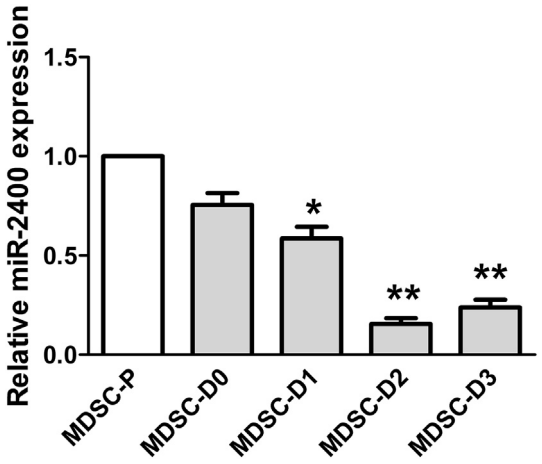

Fig. 1. Expression of miR-2400 in MDSCs during differentiation. (*$p < 0.05$, **$p < 0.01$).

transfected with pcDNA3.1 (+)-miR-2400 (Fig. 2c). However, miR-2400 inhibitors blocked the proliferation rate of MDSCs (Fig. 2 b and 2c). The IF staining assays demonstrated that transient over-expression of miR-2400 resulted in the promotion of proliferation in MDSCs, while miR-2400-I significantly attenuated the proliferation (Fig. 2d, e). Thus, our data indicated that miR-2400 was able to promote MDSCs proliferation.

3.3. MiR-2400 suppressed MYOG expression through directly targeting 3′UTR of mRNA

To explore the mechanisms of miR-2400-induced cell proliferation, we searched for the target genes of miR-2400. Bioinformatics prediction (TargetScan and PicTar) showed that the 3′UTR of the MYOG mRNA contained a conserved binding site (position of 590–597) for the seed sequence of miR-2400 (Fig. 3a). To validate whether MYOG was the direct target genes of miR-2400, a dual-luciferase reporter system was employed. We cloned 3′UTR sequences containing the predicted target site of miR-2400 or mutated sequences into the psiCHECK-2, respectively. Co-transfection of pcDNA3.1 (+)-miR-2400 with psiCHECK-2-MYOG-wt in HeLa cells showed that luciferase activity was significantly lower than those of controls ($p < 0.05$). Co-transfection pcDNA3.1 (+)-miR-2400 with psiCHECK-2- MYOG-mu did not change the luciferase activities ($p > 0.05$) (Fig. 3c). Compared with miR-2400-NC, miR-2400 inhibitors abolished the targeting 3′UTR of MYOG (Fig. 3d).

To verify whether miR-2400 affected the endogenous MYOG level, we analyzed the effects of ectopic alteration of miR-2400 in MDSCs. qRT-PCR and western blot analysis further demonstrated that over-expression of miR-2400 dramatically suppressed the endogenous mRNA and protein levels of MYOG (Fig. 3b, e, f). Furthermore, silencing of miR-2400 with its inhibitor could up-regulate the endogenous mRNA and protein levels of MYOG (Fig. 3g, h).

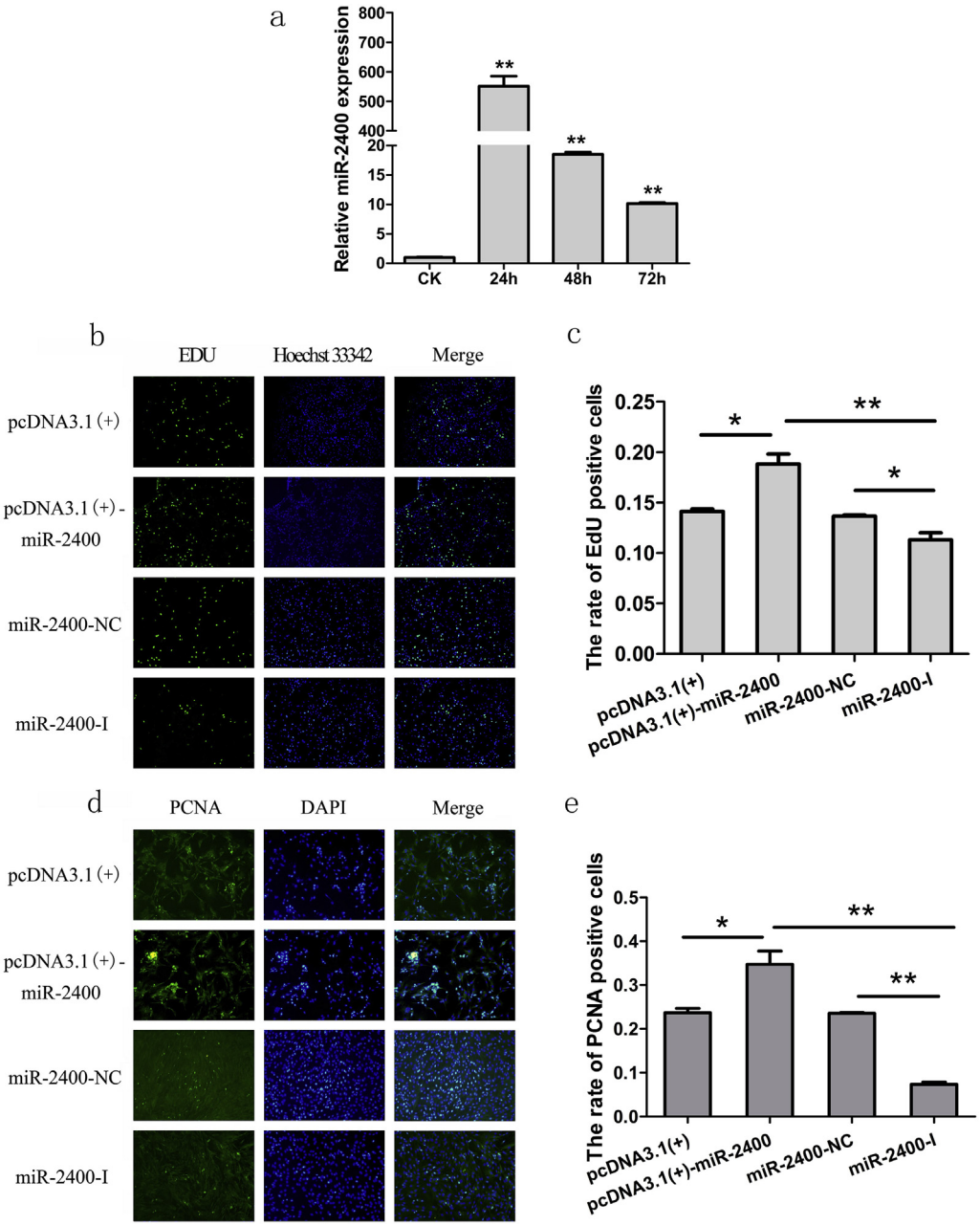

Fig. 2. MiR-2400 promotes MDSCs proliferation. (a) The expression of miR-2400 in MDSCs cells during proliferation. (b) Proliferation MDSCs cells were labeled with EdU. EdU positive cells (green). Cell nuclei (blue). (c) The percentage of EdU-positive cells. n = 6. (d) Immunofluorescence analysis of PCNA expression in MDSCs. PCNA positive cells (green). Cell nuclei (blue). (e) The percentage of PCNA-positive cells, n = 6. (NS: no significant difference, $^{*}p < 0.05$, $^{**}p < 0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. MiR-2400 was an intronic microRNA and co-expressed with its host gene

MiR-2400 is located within introns 8 of WHSC1L1 gene (Fig. 4a), it is an intronic microRNA. Bioinformatics analyses have predicted that some of intronic miRNAs are transcriptionally linked to the expression of their host gene [20], some have their own transcription regulatory elements, including promoters and terminator signals. To monitor the expression relationship between miR-2400 and WHSC1L1, their relative expression levels were first confirmed in the MDSCs and in the heart, liver, skeletal muscle and small intestine of cattle. These analyses clearly revealed that MDSCs expressed synchronous amounts of WHSC1L1 mRNA and miR-2400 (Fig. 4b). In addition, most bovine tissues showed the concordance between the expression levels of WHSC1L1 mRNA and miR-2400 (Fig. 4c).

To demonstrate the relationship between the expression of miR-2400 and WHSC1L1, the transcription of WHSC1L1 was repression by CRISPR interference (CRISPRi), which can efficiently silence transcription initiation and elongation of targeted genes. Four sgRNAs targeting different sites of WHSC1L1 promoter were designed and cloned into the pSPgRNA expression vector (pSPgRNA -N1, pSPgRNA-N2, pSPgRNA-N3 and pSPgRNA-N4) (Fig. 4d). After co-transfected with the dCas9 expression plasmid separately into MDSCs, a significantly decrease of WHSC1L1 mRNA was detected (Fig. 4e), and pSPgRNA-N2 was decreased by 88.89% compared with

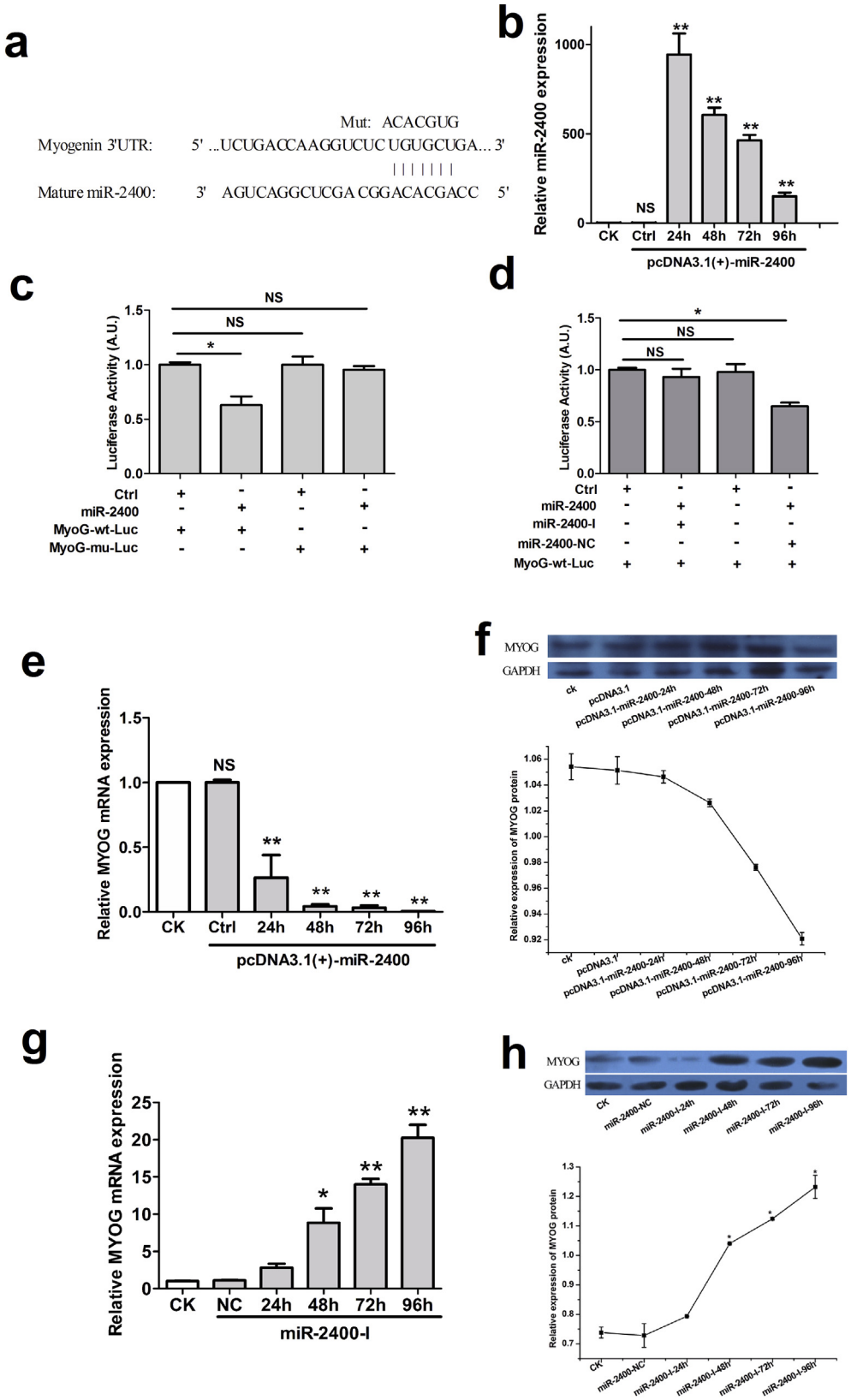

Fig. 3. MiR-2400 supresses the expression of MYOG through directly targetting 3′UTR of the mRNA. (a) Predicted miR-2400 target site in the 3′UTR of bovine MYOG. (b) The expression of miR-2400 in MDSCs cells after transfected with pcDNA3.1 (+)-miR-2400; (c) and (d) miR-2400 but not the miR-2400 mutant significantly inhibited the luciferase activities. (e) and (f). The expression of MyoG after transfected with pcDNA3.1 (+)-miR-2400; (g) and (h): The expression of MyoG after transfected with miR-2400-I. (NS: no significant difference, $^{*}p < 0.05$, $^{**}p < 0.01$).

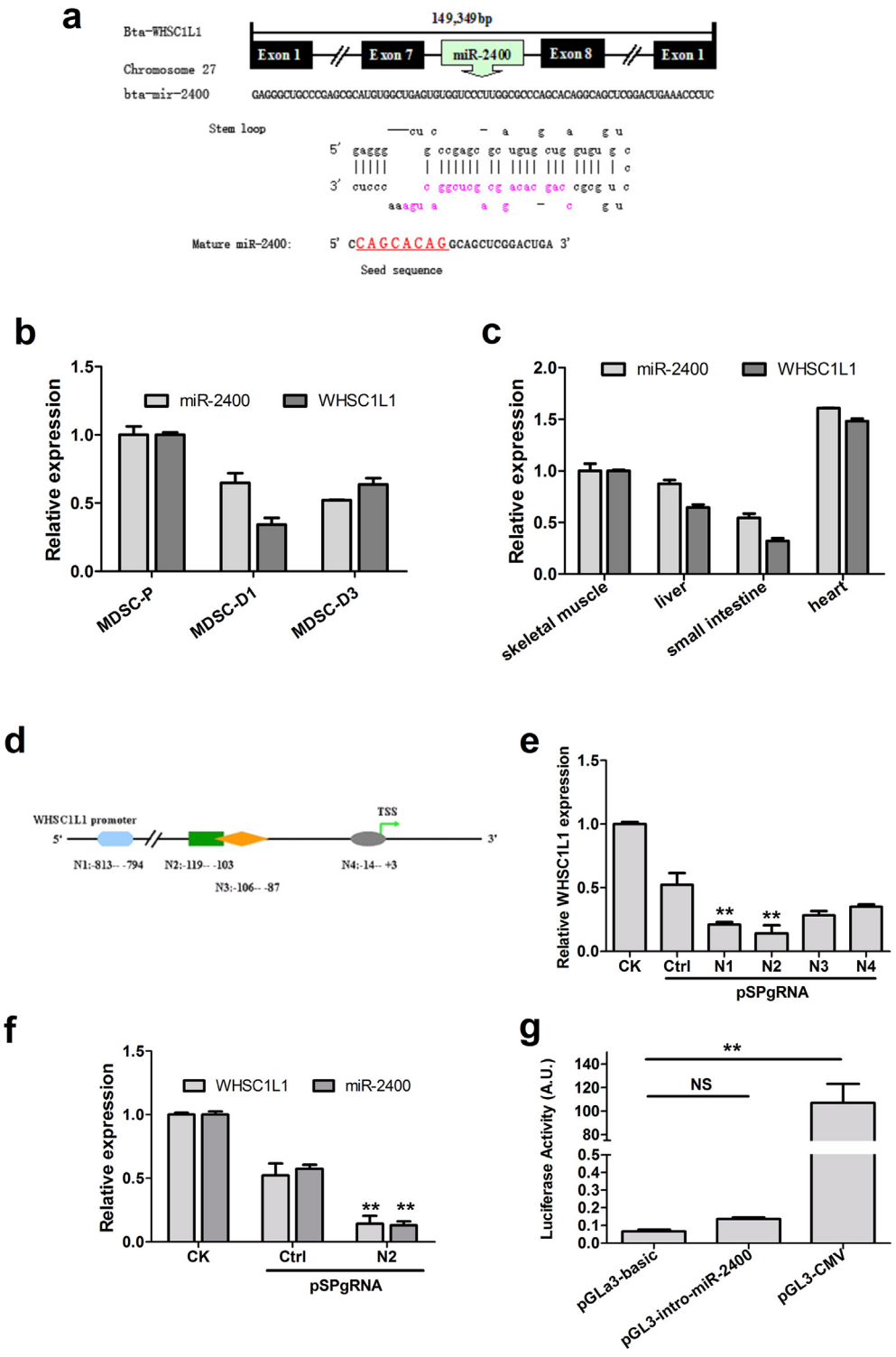

Fig. 4. MiR-2400 is coexpressed with its host gene. (a) miR-2400 is located in intron 8 of WHSC1L1 gene. Mature miR-2400 sequence comes from the 3′ arm of the precursor. Positions 2–8 of the mature miRNA are the seed region (red). (b) and (c). Expression of miR-2400 and WHSC1L1. (d) Four sgRNAs targeted different sites of WHSC1L1 promoter. (e) Expression of WHSC1L1 in MDSCs cells after co-transfected with the dCas9 expression plasmid and pSPgRNA-Nn. (f) Expression of WHSC1L1 and miR-2400 in MDSCs cells after co-transfected with the dCas9 expression plasmid and pSPgRNA-N2. (g) Luciferase activity comparison in MDSCs cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

control. Meanwhile, the expression level of miR-2400 was synchronously decreased (Fig. 4f).

To verify whether miR-2400 have its own transcriptional regulatory elements for independent expression from the intron, we performed a dual-luciferase reporter assay. Co-transfection of the pGL3-intro-miR-2400 with phRL-TK (Promega) in MDSCs showed that luciferase activity was similar with pGL3-basic ($p > 0.05$), but was significantly lower than the pGL3-CMV ($p < 0.01$) (Fig. 4g).

Together, these results showed that miR-2400 was processed from the WHSC1L1 intron rather than being transcribed as a separate RNA.

4. Discussion

The aim of this study was to explore the role and expression pattern of miR-2400 in MDSCs proliferation. More recently, studies have shown that specific miRNAs play fundamental roles during muscle proliferation and differentiation by modulating a number of transcription factors and signaling molecules [8,21–23]. By use of deep sequencing technology we found that the expression level of miR-2400 had significantly changed in MDSCs during differentiation. miR-2400 is first discovered by Glazov et al. [15] and is not identifiable in other mammalian genomes. Thus it is specific to the bovine. However, the function and expression pattern for miR-2400 in MDSCs has not been fully understood. Here, we identified that miR-2400 were down-regulated during MDSCs differentiation (Fig. 1). This result made us to speculate that miR-2400 could promote MDSCs proliferation. As expected, over-expression and inhibition studies demonstrated that miR-2400 promoted MDSCs proliferation by EdU incorporation assay and immunofluorescence staining of PCNA (Fig. 2). The precise regulatory mechanism of miR-2400 was detected by luciferase reporter assays, the results showed that miR-2400 directly targeted the 3′UTR of MYOG mRNA. Over-expression of miR-2400 in MDSCs derepressed MYOG expression and increased proliferation, whereas inhibition of miR-2400 caused persistence of MYOG protein and inhibited proliferation (Fig. 3). These data suggested that miR-2400 negatively regulated MYOG by directly targeting the 3′UTR of MYOG mRNA.

MicroRNAs are known to repress the expression of their target genes primarily by binding to the 3′UTRs of their target mRNAs [1]. Myogenin was one of target genes regulated by miR-2400. Myogenin, as a differentiation factor, is implicated in the subsequent activation of muscle-specific genes during myogenic differentiation. In the absence of myogenin, muscle differentiation does not occur either in vivo or in cell culture. Down-regulation of myogenin significantly inhibited the fusion of myoblasts [24]. Nikolaos P studies have shown that down-regulation of endogenous myogenin gene expression in terminally differentiated mouse muscle cells caused cleavage of myotubes into mononucleated cells and entered to the cell cycle [25]. MiR-186 was shown to regulate muscle differentiation and regeneration by directly targeting myogenin [26]. Our experiments showed that miR-2400 plays an important role in down-regulating the MYOG expression during MDSCs proliferation. In addition to MyoG, bioinformatics prediction showed that there are seven target genes regulated by miR-2400, such as MAGED1, MOAP1, CDK5, IFRD2, IL15 and PRDM11. It is possible that miR-2400 could promote MDSCs proliferation by targeting multiple genes, next we would go on to study the other target genes which 3′UTR contained a conserved binding site for the seed sequence of miR-2400.

MiR-2400 located at BTA27 and within introns 8 of WHSC1L1 gene. However, expression and functional aspects of intronic miRNAs are still largely unknown. It is generally believed that both host gene and miRNA share regulatory control [27–29]. miR-33 and sterol-regulatory element-binding factor-2 expression were coordinately down-regulated by cholesterol loading [30], miR-483-5p and insulin-like growth factor (Igf2) gene expression were down-regulated in mouse Hepa1-6 cells in a direct correlation by chromeceptin [31], suggesting that these gene regulatory elements are co-transcribed. We therefore compared the expression levels of miR-2400 with WHSC1L1 in MDSCs and heart, liver, skeletal muscle and small intestine of cattle, and observed that the pattern was highly similar. Then, we employed a CRISPRi system, which can efficiently repress expression of targeted genes. CRISPRi is an RNA-based method for targeted silencing of transcription in bacteria and human cells [32–34]. CRISPRi targeting is largely based on Watson-Crick base-pairing between the sgRNA and the target DNA sequence. In *E. coli*, the dCas9, when co-expressed with a sgRNA designed with a 20 bp complementary region to any gene of interest, can efficiently silence a target gene with up to 99.9% repression [19]. Using a specifically designed sgRNA, the dCas9-sgRNA complex binds to elements of WHSC1L1 promoter complementary to the sgRNA and causes a steric block that halts transcript initiation by RNA polymerase, resulting in the repression transcription of the WHSC1L1 gene. This method allowed us to experimentally show that miR-2400 is mainly processed from the WHSC1L1 intron (Fig. 4). WHSC1LI, also known as NSD3, is a member of the NSD histone methyltransferase family. Daechun K et al. studies have shown that WHSC1L1 could promote proliferation of bladder and lung cancer cell lines [35]. But the role of WHSC1L1 in regulating MDSCs proliferation is not known, it will be interesting to further investigate.

Our study provided for the first time evidence that miR-2400 gene was a novel regulator in skeletal muscle satellite cells proliferation. MyoG, as a differentiation factor during myogenic differentiation, was one of target genes of miR-2400, and possibly other target genes are regulated in order to maintain the balance between the proliferation and differentiation of MDSCs.

Acknowledgments

This work was supported by the breeding program for high-quality new varieties of genetically modified bovine from the National Major Transgenic Project [grant number 2011ZX08007-002].

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.05.112.

References

[1] V. Ambros, The functions of animal microRNAs, Nature 431 (2004) 350–355.
[2] B.P. Lewis, I.h Shih, M.W. Jones-Rhoades, D.P. Bartel, C.B. Burge, Prediction of mammalian MicroRNA targets, Cell 115 (2003) 787–798.
[3] Y. Chen, J. Gelfond, L.M. McManus, P.K. Shireman, Temporal microRNA expression during in vitro myogenic progenitor cell proliferation and differentiation: regulation of proliferation by miR-682, Physiol. Genomics 43 (2011) 621–630.
[4] W. Luo, Q. Nie, X. Zhang, MicroRNAs involved in skeletal muscle differentiation, J. Genet. Genomics 40 (2013) 107–116.
[5] Y.M. Wang, X.B. Ding, Y. Dai, X.F. Liu, H. Guo, Y. Zhang, Identification and bioinformatics analysis of miRNAs involved in bovine skeletal muscle satellite cell myogenic differentiation, Mol. Cell. Biochem. 404 (2015) 113–122.
[6] N. Motohashi, M.S. Alexander, J.C. Casar, L.M. Kunkel, Identification of a novel microRNA that regulates the proliferation and differentiation in muscle side population cells, Stem Cells Dev. 21 (2012) 3031–3043.
[7] Y. Yang, W. Sun, R. Wang, C. Lei, R. Zhou, Z. Tang, K. Li, Wnt antagonist, secreted frizzled-related protein 1, is involved in prenatal skeletal muscle development and is a target of miRNA-1/206 in pigs, BMC Mol. Biol. 16 (2015) 015–0035.
[8] J.F. Chen, E.M. Mandel, J.M. Thomson, Q.L. Wu, T.E. Callis, S.M. Hammond, F.L. Conlon, D.Z. Wang, The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation, Nat. Genet. 38 (2006) 228–233.
[9] D. Sweetman, K. Goljanek, T. Rathjen, S. Oustanina, T. Braun, T. Dalmay, A. Münsterberg, Specific requirements of MRFs for the expression of muscle specific microRNAs, miR-1, miR-206 and miR-133, Dev. Biol. 321 (2008) 491–499.
[10] C.G. Crist, D. Montarras, G. Pallafacchina, D. Rocancourt, A. Cumano, S.J. Conway, M. Buckingham, Muscle stem cell behavior is modified by microRNA-27 regulation of Pax3 expression, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 13383–13387.
[11] J. Liu, X.-j Luo, A.-w Xiong, S. Yue, M.-s Zhu, S.Y. Cheng, MicroRNA-214 promotes myogenic differentiation by facilitating exit from mitosis via down-regulation of proto-oncogene N-ras, J. Biol. Chem. 285 (2010) 26599–26607.

[12] I. Naguibneva, M. Ameyar-Zazoua, A. Polesskaya, S. Ait-Si-Ali, R. Groisman, M. Souidi, S. Cuvellier, A. Harel-Bellan, The microRNA miR-181 targets the homeobox protein Hox-A11 during mammalian myoblast differentiation, Nat. Cell Biol. 8 (2006) 278–284.
[13] L. Mi, Y. Li, Q. Zhang, C. Zhao, Y. Peng, G. Yang, X. Zheng, MicroRNA-139-5p regulates C2C12 cell myogenesis through blocking Wnt/beta-catenin signaling pathway, Biochem. Cell Biol. 93 (2015) 8–15.
[14] L. Shi, B. Zhou, P. Li, A.P. Schinckel, T. Liang, H. Wang, H. Li, L. Fu, Q. Chu, R. Huang, MicroRNA-128 targets myostatin at coding domain sequence to regulate myoblasts in skeletal muscle development, Cell Signal. 6 (2015), 00149–00147.
[15] E.A. Glazov, K. Kongsuwan, W. Assavalapsakul, P.F. Horwood, N. Mitter, T.J. Mahony, Repertoire of bovine miRNA and miRNA-like small regulatory RNAs expressed upon viral infection, PLos One 4 (2009) 0006349.
[16] S. Lee, H.S. Shin, P.K. Shireman, A. Vasilaki, H. Van Remmen, M.E. Csete, Glutathione-peroxidase-1 null muscle progenitor cells are globally defective, Free Radic. Biol. Med. 41 (2006) 1174–1184.
[17] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method, Methods 25 (2001) 402–408.
[18] M.H. Larson, L.A. Gilbert, X. Wang, W.A. Lim, J.S. Weissman, L.S. Qi, CRISPR interference (CRISPRi) for sequence-specific control of gene expression, Nat. Protoc. 8 (2013) 2180–2196.
[19] Lei S. Qi, Matthew H. Larson, Luke A. Gilbert, Jennifer A. Doudna, Jonathan S. Weissman, Adam P. Arkin, Wendell A. Lim, Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression, Cell 152 (2013) 1173–1183.
[20] A.M. Monteys, R.M. Spengler, J. Wan, L. Tecedor, K.A. Lennox, Y. Xing, B.L. Davidson, Structure and activity of putative intronic miRNA promoters, RNA 16 (2010) 495–505.
[21] C. Anderson, H. Catoe, R. Werner, MIR-206 regulates connexin43 expression during skeletal muscle development, Nucleic Acids Res. 34 (2006) 5863–5871.
[22] H.K. Kim, Y.S. Lee, U. Sivaprasad, A. Malhotra, A. Dutta, Muscle-specific microRNA miR-206 promotes muscle differentiation, J. Cell Biol. 174 (2006) 677–687.
[23] B.K. Dey, J. Gagan, A. Dutta, miR-206 and -486 induce myoblast differentiation by downregulating Pax7, Mol. Cell. Biol. 31 (2011) 203–214.
[24] S. Dedieu, G. Mazeres, P. Cottin, J.J. Brustis, Involvement of myogenic regulator factors during fusion in the cell line C2C12, Int. J. Dev. Biol. 46 (2002) 235–241.
[25] N.P. Mastroyiannopoulos, P. Nicolaou, M. Anayasa, J.B. Uney, L.A. Phylactou, Down-regulation of myogenin can reverse terminal muscle cell differentiation, PLos One 7 (2012).
[26] A. Antoniou, N.P. Mastroyiannopoulos, J.B. Uney, L.A. Phylactou, miR-186 inhibits muscle cell differentiation through myogenin regulation, J. Biol. Chem. 289 (2014) 3923–3935.
[27] S. Barik, An intronic microRNA silences genes that are functionally antagonistic to its host gene, Nucleic Acids Res. 36 (2008) 5232–5241.
[28] Y. Qi, N. Ma, F. Yan, Z. Yu, G. Wu, Y. Qiao, D. Han, Y. Xiang, F. Li, W. Wang, X. Gao, The expression of intronic miRNAs, miR-483 and miR-483*, and their host gene, Igf2, in murine osteoarthritis cartilage, Int. J. Biol. Macromol. 61 (2013) 43–49.
[29] F. Shao, X. Wang, J. Yu, H. Jiang, B. Zhu, Z. Gu, Expression of miR-33 from an SREBF2 intron targets the FTO gene in the chicken, PLos One 9 (2014).
[30] K.J. Rayner, Y. Suarez, A. Davalos, S. Parathath, M.L. Fitzgerald, N. Tamehiro, E.A. Fisher, K.J. Moore, C. Fernandez-Hernando, MiR-33 contributes to the regulation of cholesterol homeostasis, Science 328 (2010) 1570–1573.
[31] N. Ma, X. Wang, Y. Qiao, F. Li, Y. Hui, C. Zou, J. Jin, G. Lv, Y. Peng, L. Wang, H. Huang, L. Zhou, X. Zheng, X. Gao, Coexpression of an intronic microRNA and its host gene reveals a potential role for miR-483-5p as an IGF2 partner, Mol. Cell. Endocrinol. 333 (2011) 96–101.
[32] S.W. Cho, S. Kim, J.M. Kim, J.-S. Kim, Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease, Nat. Biotechnol. 31 (2013) 230–232.
[33] L. Cong, F.A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P.D. Hsu, X. Wu, W. Jiang, L.A. Marraffini, F. Zhang, Multiplex genome engineering using CRISPR/Cas systems, Science 339 (2013) 819–823.
[34] L.S. Qi, M.H. Larson, L.A. Gilbert, J.A. Doudna, J.S. Weissman, A.P. Arkin, W.A. Lim, Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression, Cell 152 (2013) 1173–1183.
[35] D. Kang, H.S. Cho, G. Toyokawa, M. Kogure, Y. Yamane, Y. Iwai, S. Hayami, T. Tsunoda, H.I. Field, K. Matsuda, D.E. Neal, B.A. Ponder, Y. Maehara, Y. Nakamura, R. Hamamoto, The histone methyltransferase Wolf-Hirschhorn syndrome candidate 1-like 1 (WHSC1L1) is involved in human carcinogenesis, Genes Chromosom. Cancer 52 (2013) 126–139.