Tissue Eng Regen Med (2017) 14(2):179–185
DOI 10.1007/s13770-017-0031-8

Online ISSN 2212-5469
Print ISSN 1738-2696

NOTE

Effects of clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) protein 9 system-Based Deletion of miR-451 in Mouse Embryonic Stem Cells on Their Self-Renewal and Hematopoietic Differentiation

Su-Jin Kim[1] · Chang-Hoon Kim[1] · Borim An[3] · Kwon-Soo Ha[4] · Seok-Ho Hong[3,5] · Kye-Seong Kim[1,2]

Received: 28 April 2016 / Revised: 17 May 2016 / Accepted: 24 May 2016 / Published online: 8 March 2017

Abstract Pluripotent stem cells (PSCs) are a useful source of cells for exploring the role of genes related with early developmental processes and specific diseases due to their ability to differentiate into all somatic cell types. Recently, the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) protein 9 system has proven to be a robust tool for targeted genetic modification. Here, we generated miR-451-deficient PSCs using the CRISPR/Cas9 system with PCR-based homologous recombination donor and investigated the impact of its deletion on self-renewal and hematopoietic development. CRISPR/Cas9-mediated miR-451 knockout did not alter the gene expressions of pluripotency, cellular morphology, and cell cycle, but led to impaired erythrocyte development. These findings propose that a combination of PSCs and CRISPR/Cas9 system could be useful to promote biomedical applications of PSCs by elucidating the function and manipulating of specific miRNAs during lineage specification and commitment.

Keywords CRISPR/Cas9 · Pluripotent stem cells · MicroRNA · Hematopoiesis

1 Introduction

Pluripotent stem cells (PSCs) hold great promise for treating various diseases and understanding early developmental processes due to their ability to self-renew indefinitely and differentiate into all somatic cell types [1, 2]. Accumulating evidence indicates that microRNAs (miRNAs), short non-coding RNAs of approximately 18-22 nucleotides, are participated in regulating self-renewal and differentiation of PSCs [3–6]. In this respect, understanding the function of miRNAs governing PSC specification and commitment into specific lineages is required for their biomedical applications, which can be accomplished by a simple, precise, and highly efficient knockout system of a miRNA of interest in PSCs.

Recently, the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) protein 9 system has proven to be a robust tool for genome editing [7–10]. This system directs the Cas9 nuclease to a

Su-Jin Kim and Chang-Hoon Kim have contributed equally to this work.

✉ Seok-Ho Hong
shhong@kangwon.ac.kr

✉ Kye-Seong Kim
ks66kim@hanyang.ac.kr

[1] Department of Biomedical Science, Graduate School of Biomedical Science and Engineering, Hanyang University, 222 Wangsimni-ro, Seongdong-gu, Seoul 04763, South Korea

[2] College of Medicine, Hanyang University, Seoul, South Korea

[3] Department of Internal Medicine, School of Medicine, Kangwon National University, Kangwondaehakgil-1, Chuncheon, Gangwon-do 24341, South Korea

[4] Department of Molecular and Cellular Biochemistry, School of Medicine, Kangwon National University, Chuncheon 24341, South Korea

[5] Stem Cell Institute, Kangwon National University, Chuncheon 24341, South Korea

desired genomic locus by a chimeric synthetic single-guide RNA (gRNA) and results in introduction of double-strand breaks for genome editing [7, 8]. Currently, CRISPR/Cas9 technique has the most attention as a simple and highly efficient knockout strategy to target specific genomic loci in various cell lines and species in comparison with other methods such as zinc finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) [7–16]. For instances, CRISPR/Cas9 system is able to target nearly any sequence in the genome by designing of gRNA. In particular, this system can eliminate the laborious processes of other methods by directly injecting RNAs encoding the Cas9 protein and gRNA into viable mouse embryos to create targeted mutant mice. In addition, mutations in multiple genes can be simultaneously achieved by injecting multiple gRNAs [17]. Recent studies reported the successful application of the CRISPR/Cas9 technique in PSCs including embryonic stem cells (ESCs) and induced PSCs (iPSCs) to reveal the role of specific mRNAs in PSC self-renewal and differentiation as well as replacement of mutant sequences with correct one [18–21]. However, few studies have been carried out to investigate the function of specific miRNAs in PSCs [22].

We previously identified erythroid lineage-specific miRNAs including miR-451 regulated during *in vitro* erythropoiesis of human umbilical cord- and human ESC-derived CD34+ cells [5]. Interestingly, miR-451 plays a negative regulatory role for erythropoiesis in Zebrafish [23]. Thus, here we generated homozygous miR-451 knockout (miR-451 KO) mouse ESC line using the CRISPR/Cas9 system with PCR-based homologous recombination donor and analyzed their self-renewal activity and hematopoietic differentiation potential. MiR-451 KO mESCs exhibited similar characteristics to its wild-type (WT) counterpart regarding the gene expressions of pluripotency, cellular morphology, and cell cycle. Interestingly, we found that miR-451 KO mESCs gave rise to much more hematopoietic progenitor cells (HPCs). However, we observed significant defect of miR-451 KO mESC-derived HPCs in generating blast forming unit-erythrocyte (BFU-E). Our findings suggest that combination of PSCs and CRISPR/Cas9 system could be useful to promote biomedical applications of PSCs and understand the intricate regulatory mechanisms of PSC differentiation by elucidating the function of specific miRNAs during lineage specification and commitment.

2 Materials and methods

2.1 Design of the double nickase system based on Cas9 D10A nickase and PCR-based homologous recombination donors

The target sequences present in gRNA were selected by the Cas9 design tools (http://crispr.mit.edu/) by Feng Zhang's group and assembled into all-in-one Cas9 D10A nickase vector (Add gene Plasmid #48141; Feng Zhang). Assembled nickases were transfected to wild-type CCE cells to monitor nickase activity. We selected the most efficient nickase pairs based on T7 Endonuclease I assay. To delete genomic regions of miR-451 gene through homologous recombination (HR) with an antibiotic puromycin cassette, we designed the primers containing total 190 bp of gene-specific homologous sequences (160 bp) with amplifying gene sequences (30 bp) for the PGK-Puro cassette using pLenti GFP Puro (Fig. 1A, Add gene Plasmid #17448) as a template. miR451-N arm: ATATACTGTAAGTTTGTGA TGAGACACTACAGTATAGATGATGTACTAGTCTGG GTACCCCACCTCCAGAGCCTGCCTGGTTTGCAGCA GAGATGCAGAAGTACACGGGCTCACTGCTCGGCCT AATCAAGCCTGCTGACAGCTGTGGCACTTGGGAA TGGAATTCTACCGGGTAGGGGAGGCGCTTTTC. miR451-C arm: GAAAGGAGCGCACGACCCCATGCATCTCG ACTGAGTTTAGTAATGGTAACGGTTCTCTTGCTGCTC CCACAAACTGTGCCAAGAAGAGCTCATGACCCTGGA GCAGACTGCTGGAAGAAAAGGACACCCAGGCTGAC AAGAGAATGGGGTTGGGGGAAAGGGTACATTTTCCT CTTCACTGTGCCAAAGA.

2.2 Cell culture and DNA transfection

CCE mESCs were maintained on irradiated mouse embryonic fibroblast (MEF) in DMEM containing 15% fetal bovine serum (FBS; Hyclone), 10^3 unit/ml leukemia inhibitory factor (LIF, Merck Millipore), 0.1 mM β-mercaptoethanol (Gibco), 1% non-essential amino acids (NEAA; Gibco), 1x penicillin/streptomycin, and 1x GlutaMax (Gibco). Cells were passaged every 2–3 days and changed with fresh medium every other day. DNA transfection was performed with lipofectamine 2000 (Invitrogen).

2.3 Establishment of miR-451 KO mESC lines

PCR-based homologous recombination donor and two nickases were transfected to parental CCE cells and were selected with 3 g/ml puromycin (Sigma) for a week. The puromycin-resistant colonies were isolated and cultured with 1 μg/ml puromycin further.

2.4 Verification of miR-451 KO mESC lines by genomic sequencing and RT-PCR

Genomic DNA was extracted using a rapid method [24]. The samples were suspended in 500 μl lysis buffer [10 mM Tris (pH 8.0), 10 mM EDTA, and 2% SDS], and 50 μl 10% SDS was added followed by 5–10 μl 20 mg/ml proteinase K. The samples were incubated for 1 h at 56°C. Genomic

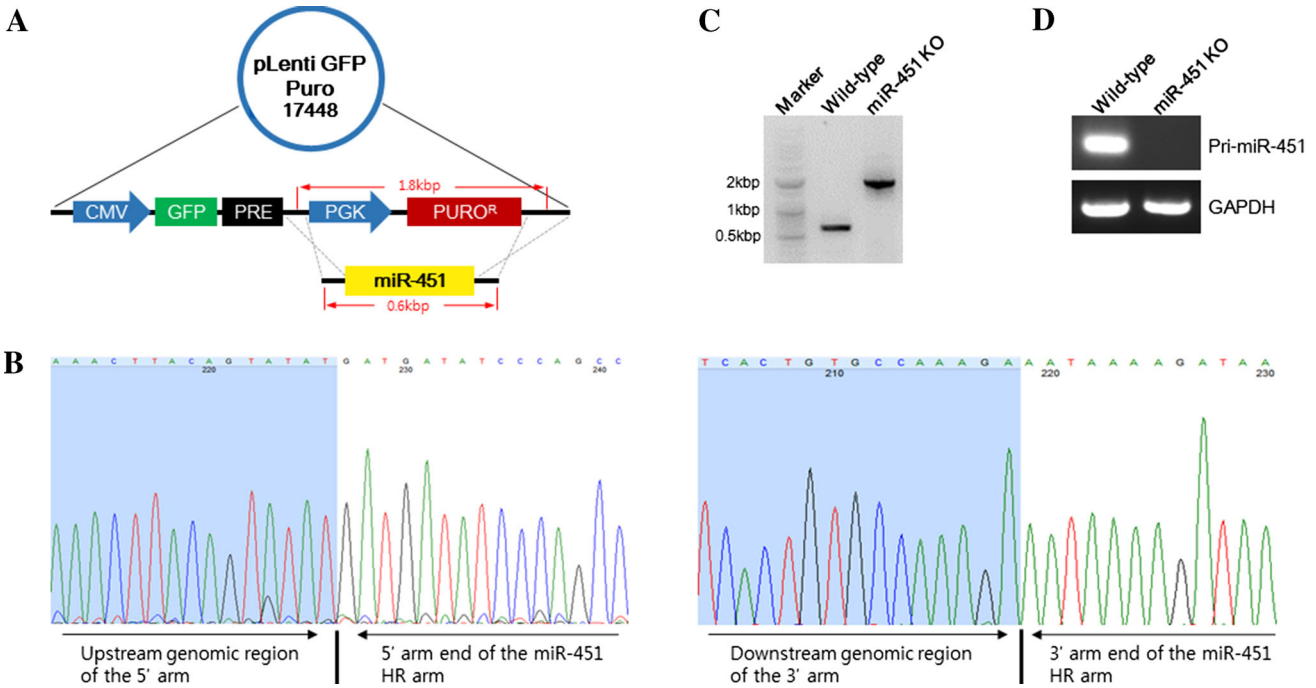

Fig. 1 Knockout of the miR-451 by CRISPR-Cas9 nickase. **A** Design of homologous recombination (HR) arms for the miR-451 using pLenti GFP Puro and nickase. **B** Confirmation of the site-specific recombination of the HR arms into miR-451 gene by DNA sequencing. **C** Site-specific double chromosomal recombination of the HR arms in CCE mESCs. **D** RT-PCR analysis to confirm the knockout of miR-451

DNA was isolated using mini-prep DNA column. To identify HR donor insertion by homologous recombination, 5' and 3' gene-specific primers were chosen from the adjacent upstream and downstream of the HR destination site. Genomic PCR was performed using EF-taq (Solgent). RT-PCR primers are Forward primer: ACA GGA TCA GGG AGA TGC TGC T, Reverse: ACT CTG ACT GGC TGG CTA TCT CTA. All genomic PCR products were analyzed by sequencing.

2.5 BrdU assay

WT and miR-451 KO mESCs were treated with 10 μM of bromodeoxyuridine (BrdU) for 4 h before being harvested and then fixed in 70% ethanol. Cells were stained with allophycoerythrin (APC)-conjugated anti-BrdU antibody and 7-amino actinomycin (7ADD) using APC-BrdU Flow Kit (BD Pharmingen). Flow cytometric analysis was performed by using FACSCantoII flow cytometer (BD Biosciences) and resultant data were analyzed by the FlowJo software (Tree Star).

2.6 Hematopoietic differentiation

Prior to the embryoid body (EB) formation, WT and miR-451 KO mESCs were pre-differentiated in IMDM medium with 10 ng/ml of LIF. The pre-differentiated cells were replated in bacterial petri dished with 1,500 cells/ml concentration. On the following day, EBs were resuspended in StemPro34 media (Life Technologies) supplemented with StemPro34 supplement, 2 mM L-glutamine (Life Technologies), 50 μg/mL L-ascorbic acid (Sigma Aldrich), 200 μg/mL transferrin (Sigma Aldrich), 4×10^{-4} M monothioglycerol (Life Technologies) and 4 ng/mL BMP4 (R&D systems). After 2.5 days, the EBs were treated with 5 ng/mL FGF2 (Life Technologies), 5 ng/mL Activin A (R&D systems), 5 ng/mL VEGF (R&D systems), 20 ng/mL TPO (R&D systems) and 100 ng/mL SCF (R&D systems) for 4-8 days. The colony forming unit (CFU) assay was performed by plating single cells (25,000 cells per well of 24-well non-tissue culture plate) in methylcellulose (M3434, Stem Cell Technologies, Inc). The colonies were scored after 10 days.

2.7 Flow cytometry

Single cell suspensions were harvested from mESC cultures by dissociation with 0.05% trypsin-EDTA and resuspended in 1% FBS-PBS. The cells were filtered through a 70μm cell strainer and incubated for 30 min at 4°C with CD41-fluorescein-isothiocyanate (FITC) and CD117-APC antibodies. After staining, the cells were rinsed once and stained with 7AAD to exclude dead cells. For intracellular staining of Oct4, Sox2 and Nanog, the

Cytofix/Cytoperm buffer (BD Biosciences) was used according to the manufacturer's instructions. All antibodies were purchased from BD Biosciences. Flow cytometric analysis was performed by using FACSCanto II flow cytometer and acquired data were analyzed by the FlowJo software.

2.8 Statistical analysis

Values for all measurements were presented as mean± SD. Statistical significance was determined using Student's *t*-test and $p<0.05$ was considered statistically significant.

3 Results

3.1 Establishment of miR-451 KO ESCs by CRISPR/Cas9 nickase with HR donor

To remove miR-451 gene in parental CCE mESCs, we used the Cas9 D10A nickase pair for reducing the off-target effects of wt Cas9 nuclease [25]. Usually, genetic exchange takes place between a pair of homologous DNA sequences [26]. We therefore designed the HR donor arms containing 150bp gene-specific sequences that occur in the HR event between identical miR-451 genomic region and amplified PGK-Puro HR cassette (Fig. 1A). As anticipated, nickase pairs with HR donor can lead to precise homologous recombination event based on our sequencing data (Fig. 1B, C). In addition, pri-miR-451 transcripts were not expressed in miR-451 KO mESCs (Fig. 1D).

3.2 Analysis of the effects of miR-451 deficiency in mESCs

We first asked if miR-451 deficiency causes the defects in the self-renewal and proliferation of mESCs. WT and miR-451 KO mESCs were maintained for long term (more than 10 passages) under same undifferentiated condition and did not show any significant changes to their colony morphology (Fig. 2A). Both cell types exhibited typical cellular morphology of undifferentiated ESCs. We then investigated the expression of pluripotency genes in WT and miR-451 KO mESCs. A major proportion of both WT and miR-451 KO mESCs were positive for Oct4 (81.7±1.4 vs. 82.6±0.5), Nanog (98.5±0.2 vs. 96.1±1.1), and Sox2 (94.3±1.8 vs. 90.1±1.5) (Fig. 2B). To determine whether miR-451 is associated with proliferative potential and specific phases of the cell cycle, we performed a BrdU assay where the cells were exposed to BrdU for 4 hrs prior to cell counting and measuring the cell cycle distribution. Knockout of miR-451 had no significant effect on the cellular proliferation (Fig. 2C). We also found a similar proportion of G0/G1, S and G2/M phases between two cell types (Fig. 2 D). These results indicate that miR-451 is not critical for maintenance of undifferentiated state and proliferation of mESCs.

3.3 MiR-451 is involved in regulating early hematopoiesis

To determine if miR-451 is associated with differentiation potential, we generated EBs from WT and miR-451 KO mESCs and cultured for 10 days under established conditions for hematopoietic differentiation [27]. There was no significant difference in EB formation and shape between two cell types during hematopoietic differentiation (Fig. 3A). At days 6, 8 and 10 of hematopoietic differentiation, dissociated EBs were analyzed by flow cytometry to determine the frequency of definitive hematopoietic cells (CD41+) and hematopoietic progenitor cells (HPCs, CD41+CD117+). There was no significant difference in the frequency of CD41+ and CD41+CD117+ cells between two cell types at day 6 of hematopoietic differentiation. However, the frequencies of CD41+ and CD41+CD117+ cells were significantly increased in miR-451 KO-derived EBs compared to WT-derived EBs at days 8 and 10 (Fig. 3B, C). We further conducted a functional CFU assay to compare multilineage hematopoietic progenitor potential derived from WT- and miR-451 KO-derived EBs. While no difference in the number of CFU-GM, miR-451 KO-derived HPCs were defective in generating BFU-E colony compared to WT-derived HPCs (Fig. 3D). These results suggest that early hematopoietic and erythropoietic development can be regulated by modulating of miR-451 expression.

4 Discussion

The understanding of the regulatory mechanism of miRNAs in self-renewal and differentiation of PSCs is of crucial importance for their basic and clinical use. RNA interference (RNAi) technology including short-interfering RNA and short-hairpin RNA to reduce expression of specific miRNAs have been widely used *in vitro* and *in vivo* [28]. However, these methods have several limits to completely knockout of miRNAs due to their low efficiency and undesirable off-target effects. Recent advanced gene editing technique, called CRISPR/Cas9 system has some advantages over other methods such as RNAi, ZFN, and TALEN and currently has the most attention as a simple and highly efficient genome editing strategy to target specific genomic loci in various cell types and species [7–13, 15, 16]. Here, we employed the CRISPR/Cas9 system to generate miR-451 KO mESC clones and

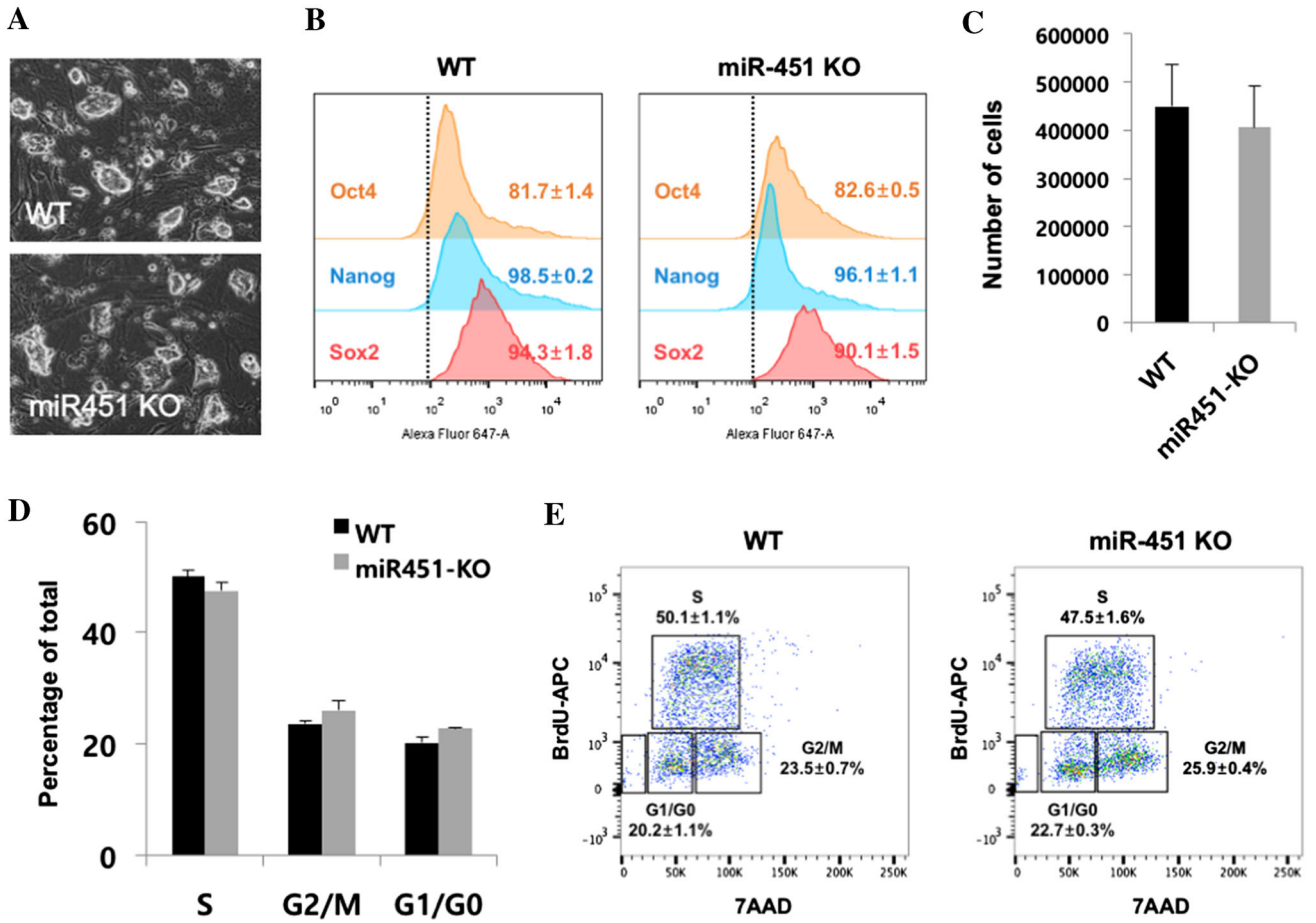

Fig. 2 Knockout of miR-451 does not change undifferentiated state and proliferative activity of mESCs *in vitro*. **A** Representative morphology of undifferentiated WT and miR-451 KO mESCs growing on irradiated MEFs. **B** Percentage of Oct4, Nanog and Sox2 in WT and miR-451 KO mESC cultures by flow cytometry. Frequencies shown in histograms indicate the average and standard deviation. **C** Knockout of miR-451 did not affect on proliferative activity. **D**, **E** Changes of cell cycle compartments (S, G1/G0 and G2/M phases) were measured by BrdU assay. Frequencies shown in FACS plots indicate the average and standard deviation. *Error bars* denote SD for three independent experiments

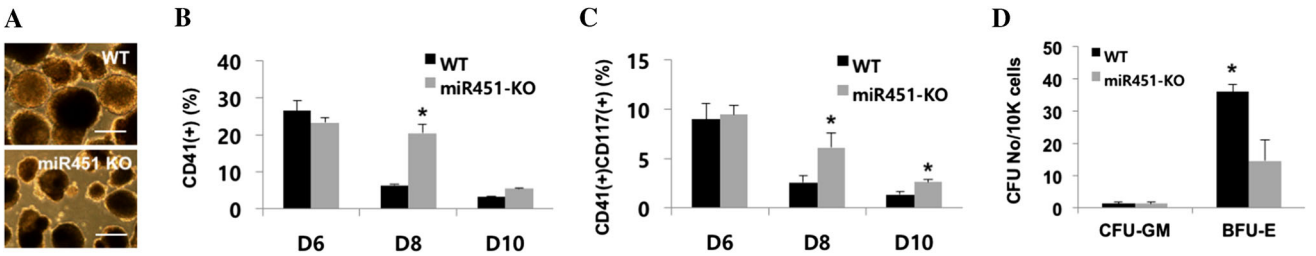

Fig. 3 Effect of miR-451 KO on hematopoietic differentiation *in vitro*. **A** Representative morphology of WT- and miR-451 KO-derived EBs growing under hematopoietic condition. *Bars*, 100 μm. **B**, **C** Flow cytometry analysis for CD41 and CD 117 expression at day 6, 8 and 10 day of hematopoietic differentiation. **D** Knockout of miR-451 is defective in generating BFU-E colony. *Error bars* denote SD for three independent experiments. *$p<0.05$

elucidated that miR-451 is associated with early hematopoietic and erythroid differentiation from mESCs *in vitro*. To our best knowledge, it is the first report to demonstrate the role of specific miRNA during PSC-derived hematopoiesis using CRISPR/Cas9 system. Our study proposes that combination of PSCs and CRISPR/Cas9 system could be useful to modulate specific miRNAs for understanding their regulatory mechanisms in early embryonic development and promoting clinical applications of PSCs.

Previous studies have reported the crucial function of miR-451 in erythroid differentiation, which is conserved among several species. In wild-type zebrafish embryos, morpholino knockdown of miR-451 has no effects on initiation of early erythroid differentiation, but retards erythrocyte maturation [23]. Overexpression of miR-451 mutant zebrafish embryos partially rescues erythrocyte maturation via targeting of gata-2 mRNA [29]. Similarly, transplantation of mouse HPCs transduced with miR-451 inhibitory lentiviral constructs into mouse bone marrow (BM) displayed markedly reduced Ter119+ erythroblast cells in the BM [30]. Several groups have generated miR-451 knockout mice, which exhibited a reduction in hematocrit and impairment of late erythroblast maturation in BM and spleen [31–33]. It has been also reported that miR-451 is involved in promoting erythroid maturation of HPCs (CD133+ or CD34+ cells) derived from human umbilical cord and peripheral blood via strong induction of β-globin expression [34–36]. Consistent with previous findings, our study showed that miR-451 deficiency in mESCs results in reduction of an early erythroid progenitor subset that contains to BFU-E cells. Moreover, we found that miR-451 KO in mESCs led to a significant increase of early HPC populations (CD41+ and CD41+CD117+), suggestive of miR-451 as a potential erythrocytic inducer during hematopoietic differentiation.

Taken together, our study elucidates that miR-451 is essential for early hematopoietic and erythroid differentiation from mESCs and suggests that CRISPR/Cas9-based genomic editing technique could be a highly efficient and robust tool for determining the role of specific miRNA and genetically manipulating cell fate determination of PSCs. Further studies will be needed to reveal the detailed regulatory mechanism of miR-451 in PSC hematopoietic differentiation.

Acknowledgements This study was supported by a grant from the Medical Research Center (2008-0062287) and the Basic Research Lab Program (2015R1A4A1038666) funded by the NRF of the Ministry of Science, ICT & Future Planning, Republic of Korea.

Compliance with ethical standards

Conflict of interest The authors have no financial conflicts of interest.

Ethical statement There are no human or animal experiments carried out for this article.

References

1. Romito A, Cobellis G. Pluripotent stem cells: current understanding and future directions. Stem Cells Int. 2016. doi:10.1155/2016/9451492
2. Ireland RG, Simmons CA. Human pluripotent stem cell mechanobiology: manipulating the biophysical microenvironment for regenerative medicine and tissue engineering applications. Stem Cells. 2015;33:3187–96.
3. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281–97.
4. Lee YJ, Ramakrishna S, Chauhan H, Park WS, Hong SH, Kim KS. Dissecting microRNA-mediated regulation of stemness, reprogramming, and pluripotency. Cell Regen (Lond). 2016;5:2.
5. Jin HL, Kim JS, Kim YJ, Kim SJ, Broxmeyer HE, Kim KS. Dynamic expression of specific miRNAs during erythroid differentiation of human embryonic stem cells. Mol Cells. 2012;34:177–83.
6. Bhin J, Jeong HS, Kim JS, et al. PGC-enriched miRNAs control germ cell development. Mol Cells. 2015;38:895–903.
7. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339:819–23.
8. Mali P, Yang L, Esvelt KM, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339:823–6.
9. Wang H, Yang H, Shivalila CS, et al. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell. 2013;153:910–8.
10. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157:1262–78.
11. Heo YT, Quan X, Xu YN, et al. CRISPR/Cas9 nuclease-mediated gene knock-in in bovine-induced pluripotent cells. Stem Cells Dev. 2015;24:393–402.
12. Renneville A, Van Galen P, Canver MC, et al. EHMT1 and EHMT2 inhibition induces fetal hemoglobin expression. Blood. 2015;126:1930–9.
13. Ru R, Yao Y, Yu S, et al. Targeted genome engineering in human induced pluripotent stem cells by penetrating TALENs. Cell Regen (Lond). 2013;2:5.
14. Yan Q, Zhang Q, Yang H, et al. Generation of multi-gene knockout rabbits using the Cas9/gRNA system. Cell Regen (Lond). 2014;3:12.
15. Zhu S, Rong Z, Lu X, Xu Y, Fu X. Gene targeting through homologous recombination in monkey embryonic stem cells using CRISPR/Cas9 system. Stem Cells Dev. 2015;24:1147–9.
16. Huang K, Du J, Ma N, et al. GATA2(–/–) human ESCs undergo attenuated endothelial to hematopoietic transition and thereafter granulocyte commitment. Cell Regen (Lond). 2015;4:4.
17. Harrison MM, Jenkins BV, O'Connor-Giles KM, Wildonger J. A CRISPR view of development. Genes Dev. 2014;28:1859–72.
18. Liao J, Karnik R, Gu H, et al. Targeted disruption of DNMT1, DNMT3A and DNMT3B in human embryonic stem cells. Nat Genet. 2015;47:469–78.
19. Song B, Fan Y, He W, et al. Improved hematopoietic differentiation efficiency of gene-corrected beta-thalassemia induced pluripotent stem cells by CRISPR/Cas9 system. Stem Cells Dev. 2015;24:1053–65.
20. Tan EP, Li Y, Velasco-Herrera Mdel C, Yusa K, Bradley A. Off-target assessment of CRISPR-Cas9 guiding RNAs in human iPS and mouse ES cells. Genesis. 2015;53:225–36.
21. Zhou HY, Katsman Y, Dhaliwal NK, et al. A Sox2 distal enhancer cluster regulates embryonic stem cell differentiation potential. Genes Dev. 2014;28:2699–711.
22. Ma Y, Yao N, Liu G, et al. Functional screen reveals essential roles of miR-27a/24 in differentiation of embryonic stem cells. EMBO J. 2015;34:361–78.
23. Dore LC, Amigo JD, Dos Santos CO, et al. A GATA-1-regulated microRNA locus essential for erythropoiesis. Proc Natl Acad Sci USA. 2008;105:3333–8.
24. Ghatak S, Muthukumaran RB, Nachimuthu SK. A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. J Biomol Tech. 2013;24:224–31.
25. Ran FA, Hsu PD, Lin CY, et al. Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell. 2013;154:1380–9.

26. Sagi D, Tlusty T, Stavans J. High fidelity of RecA-catalyzed recombination: a watchdog of genetic diversity. Nucl Acids Res. 2006;34:5021–31.
27. Lesinski DA, Heinz N, Pilat-Carotta S, et al. Serum- and stromal cell-free hypoxic generation of embryonic stem cell-derived hematopoietic cells in vitro, capable of multilineage repopulation of immunocompetent mice. Stem Cells Transl Med. 2012;1: 581–91.
28. Fennell M, Xiang Q, Hwang A, et al. Impact of RNA-guided technologies for target identification and deconvolution. J Biomol Screen. 2014;19:1327–37.
29. Pase L, Layton JE, Kloosterman WP, Carradice D, Waterhouse PM, Lieschke GJ. miR-451 regulates zebrafish erythroid maturation in vivo via its target gata2. Blood. 2009;113: 1794–804.
30. Papapetrou EP, Korkola JE, Sadelain M. A genetic strategy for single and combinatorial analysis of miRNA function in mammalian hematopoietic stem cells. Stem Cells. 2010;28:287–96.
31. Patrick DM, Zhang CC, Tao Y, et al. Defective erythroid differentiation in miR-451 mutant mice mediated by 14-3-3zeta. Genes Dev. 2010;24:1614–9.
32. Rasmussen KD, Simmini S, Abreu-Goodger C, et al. The miR-144/451 locus is required for erythroid homeostasis. J Exp Med. 2010;207:1351–8.
33. Yu D, dos Santos CO, Zhao G, et al. miR-451 protects against erythroid oxidant stress by repressing 14-3-3zeta. Genes Dev. 2010;24:1620–33.
34. Kouhkan F, Soleimani M, Daliri M, et al. miR-451 up-regulation, induce erythroid differentiation of CD133+ cells independent of cytokine cocktails. Iran J Basic Med Sci. 2013;16:756–63.
35. Zhan M, Miller CP, Papayannopoulou T, Stamatoyannopoulos G, Song CZ. MicroRNA expression dynamics during murine and human erythroid differentiation. Exp Hematol. 2007;35:1015–25.
36. Masaki S, Ohtsuka R, Abe Y, Muta K, Umemura T. Expression patterns of microRNAs 155 and 451 during normal human erythropoiesis. Biochem Biophys Res Commun. 2007;364:509–14.