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Dissecting the Roles of miR-302/367 Cluster in Cellular Reprogramming Using TALE-based Repressor and TALEN

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SUMMARY

MicroRNAs are important gene regulators involved in many biological processes, including stemness maintenance and cellular reprogramming. Current methods used in loss-of-function studies of microRNAs mainly include locked nucleic acid (LNA) oligonucleotides and miRZip inhibitors, which have several limitations. Due to their unique gene structures and small sizes, there is no efficient or simple strategy to knock down or knock out microRNAs or whole microRNA clusters. Here, we demonstrate knockdown of the miR-302/367 cluster by using the Kruppel-associated box repressor domain fused with specific transcription activator-like effectors (TALEs) designed to bind the miR-302/367 cluster promoter. We also designed two pairs of TALE nucleases (TALENs) to efficiently delete the miR-302/367 cluster in primary human fibroblasts and determined that knockout of the miR-302/367 cluster completely blocked induced pluripotent stem cell (iPSC) generation. Together, our results demonstrate that TALE-based transcriptional repressor and TALENs are two promising approaches for loss-of-function studies of microRNA clusters in somatic cells and pluripotent stem cells.

INTRODUCTION

MicroRNAs (miRNAs) are small 18–24 nt single-stranded noncoding RNA molecules that regulate gene expression at the posttranscriptional level. These small RNAs bind to partially complementary target sites in messenger RNAs (mRNAs), leading to degradation of target mRNAs or translational repression of encoded proteins (Bartel, 2004). To date, there are 1281 and 2042 mature miRNAs in mouse and human genomes, respectively. These miRNAs are implicated in many biological processes, diseases, development, and cellular reprogramming (Bartel, 2004; Johnston and Hobert, 2003).

Several approaches for knockdown of miRNAs have been extensively used, such as locked nucleic acid (LNA) oligonucleotides, antagomirs, and miRZip inhibitors (Liao et al., 2011; Robertson et al., 2010; Xia et al., 2012). Unfortunately, LNA and antagomirs can only transiently inhibit miRNA function. In contrast, miRZips are stably expressed RNAi hairpins and can permanently inhibit miRNA function; however, their inhibitory efficiency, similar to that of LNA and antagomir, is dependent on their dosage in each cell. In addition, the specificity of these three miRNA inhibitors is inversely proportional to their dosage delivered in cells. Therefore, there are significant concerns regarding the specificity and potency of these miRNA inhibitors (Robertson et al., 2010).

To overcome these obstacles, we describe two approaches for studying miRNA functions: (1) TALE-based transcriptional repressor for knockdown of miRNA clusters, and (2) TALENs for knockout of miRNA clusters. Originally found to be secreted by *Xanthomonas* and *Ralstonia* bacteria, TALE is protein consisting of multiple repeated, highly conserved 33–34 amino acid sequences (Moscou and Bogdanove, 2009), which can be quickly engineered to bind virtually any desired DNA sequence. Thus, TALE can regulate expression of endogenous genes when tethered with transcription activator or repressor domains and edit the genome when fused with the FokI cleavage domain. TALEN is an emerging technology for genome editing (Hockemeyer et al., 2011).

In the current study, we applied these two approaches to study the roles of the endogenous miR-302/367 cluster in cellular reprogramming. The miR-302/367 cluster is a polycistronic miRNA consisting of five mature miRNAs (miR-302b/c/a/d and miR-367). It has been well documented that overexpression of this cluster promotes cellular reprogramming and maintains the stemness of human embryonic stem cells (hESCs) (Lin et al., 2011; Miyoshi et al., 2011; Rosa et al., 2009). However, it remains unknown whether the endogenous miR-302/367 cluster is required for the generation of induced pluripotent stem cells (iPSCs). In this study, we efficiently knocked down the expression of mature miR-302/367 miRNAs using the TALE-based transcriptional repressor and deleted the entire

miRNA cluster by TALENs to investigate the roles of this cluster in cellular reprogramming.

RESULTS

Knockdown of the Endogenous miR-302/367 Cluster by TALE Transcriptional Repressor

Previous studies reported that the miR-302/367 cluster is transcribed by RNA polymerase II and contains a 5′ cap and a polyadenylated tail, and it is subjected to transcriptional regulation by transcription factors such as OCT4 and SOX2 (Card et al., 2008; Liu et al., 2011; Rosa and Brivanlou, 2011). Therefore, we designed two TALEs that recognize specific sequences within the human miR-302/367 promoter region and fused each TALE with the Kruppel-associated box (KRAB) transcriptional repressor domain (Cong et al., 2012; Margolin et al., 1994) (Figure 1A). To examine inhibitory function of the designed miR-302/367 cluster-specific TALE-KRAB constructs (TALE1-KRAB and TALE2-KRAB), we generated a luciferase reporter driven by the promoter of the miR-302/367 cluster and then cotransfected it into 293T cells, together with lentiviral expression vectors expressing TALE1-KRAB or TALE2-KRAB. The results revealed that both TALE1-KRAB and TALE2-KRAB repressed the luciferase activity of the miR-302/367 reporter more than 40-fold compared to the TALE-control group (Figure 1B). Since both TALE-KRAB constructs had comparable inhibitory activity, we selected TALE1-KRAB for the following studies.

To determine if TALE1-KRAB is capable of downregulating expression of the miR-302/367 cluster, we used lentiviruses expressing TALE1-KRAB or TALE-control to infect hESCs (H1 line) that express a high level of the miR-302/367 cluster. We sorted GFP^+ infected cells and measured the transcripts of primary (pri)-miRNA302/367 cluster by quantitative PCR (qPCR). Our qPCR analysis showed that TALE1-KRAB indeed repressed expression of the miR-302/367 cluster by 80% more than the TALE-control (Figure 1C).

The miR-302/367 cluster is a target of hESC core factors OCT4 and SOX2, and it is induced in cellular reprogramming (Card et al., 2008; Lin et al., 2011; Liu et al., 2011; Miyoshi et al., 2011; Rosa and Brivanlou, 2011; Rosa et al., 2009). Therefore, we decided to assess the ability of TALE1-KRAB to reduce the expression of the miR-302/367 cluster during reprogramming. To do so, we infected human foreskin fibroblasts (HFFs) with retroviruses expressing the four human reprogramming factors OKSM (Zhang et al., 2011) and lentiviruses expressing TALE1-KRAB. Three days after the first infection, we analyzed the expression levels of pri-miR-302/367 by semiquantitative PCR as well as the expression levels of five mature miR-302/367 miRNAs by qPCR. As expected, the transcripts of pri-miR-302/367 were decreased 70% by TALE1-KRAB (Figure 1D), and the expression levels of miR-302b, c, a, d, and miR-367 were reduced by 70%–90% more in HFFs expressing TALE1-KRAB than in the TALE-control (Figures 1E–1I). In addition, our qPCR analysis showed that the expression of *LARP7* and *C4ORF21*, the two genes near the miR-302/367 cluster, was not significantly altered by TALE1-KRAB (Figure 1J). Thus, this result suggests that knockdown of the miR-302/367 cluster by TALE-based repressor had a minimal effect on expression of its neighboring genes.

Knockdown of the miR-302/367 Cluster by TALE1-KRAB Impairs Cellular Reprogramming

To assess the effects of miR-302/367 knockdown on iPSC generation, we performed a reprogramming assay to compare the reprogramming efficiency among four groups of HFFs: (1) wild-type HFFs, (2) HFFs infected with lentivirus expressing TALE control, (3) HFFs infected with lentivirus expressing TALE1-KRAB, and (4) HFFs transduced with both lentivirus expressing TALE1-KRAB and retrovirus expressing miR-302/367 cluster (Figure 2A). After 4 weeks of standard reprogramming of cell cultures, we found that HFFs expressing TALE-control had comparable reprogramming efficiency as wild-type HFFs. In contrast, knockdown of the miR-302/367 cluster by TALE1-KRAB resulted in an ~3- to 4-fold decrease in the number of $TRA\text{-}1\text{-}60^+$ iPSC colonies. Notably, forced expression of the miR-302/367 cluster rescued the reprogramming deficiency of HFFs expressing TALE1-KRAB. We performed the same reprogramming assays using two other types of primary cells (skin fibroblasts and hepatocytes) and observed similar results (Figures 2B and 2C). Taken together, our data showed that TALE1-KRAB efficiently repressed the expression of miR-302/367 miRNAs and the reduced expression of this cluster impaired cellular reprogramming efficiency. This result is consistent with a previous report showing suppressed reprogramming of mouse somatic cells by miR-302-specific antagomirs (Liao et al., 2011).

To demonstrate that TALE-KRAB is a useful approach for knockdown of miRNA cluster, we designed a specific TALE-KRAB to target the human let-7a-1/let-7f-1/let-7f cluster (Wang et al., 2011) (Figure 3A). Our data showed that this specific TALE-KRAB reduced the expression of three miRNA members transcribed from this cluster by ~80%–90% (Figure 3B), suggesting that TALE-KRAB could be used as a general approach for knockdown of miRNA clusters.

Knockout of the Whole miR-302/367 Cluster by Two Pairs of Specific TALENs

Although our data show that TALE1-KRAB provides an effective approach for knockdown of miRNA clusters

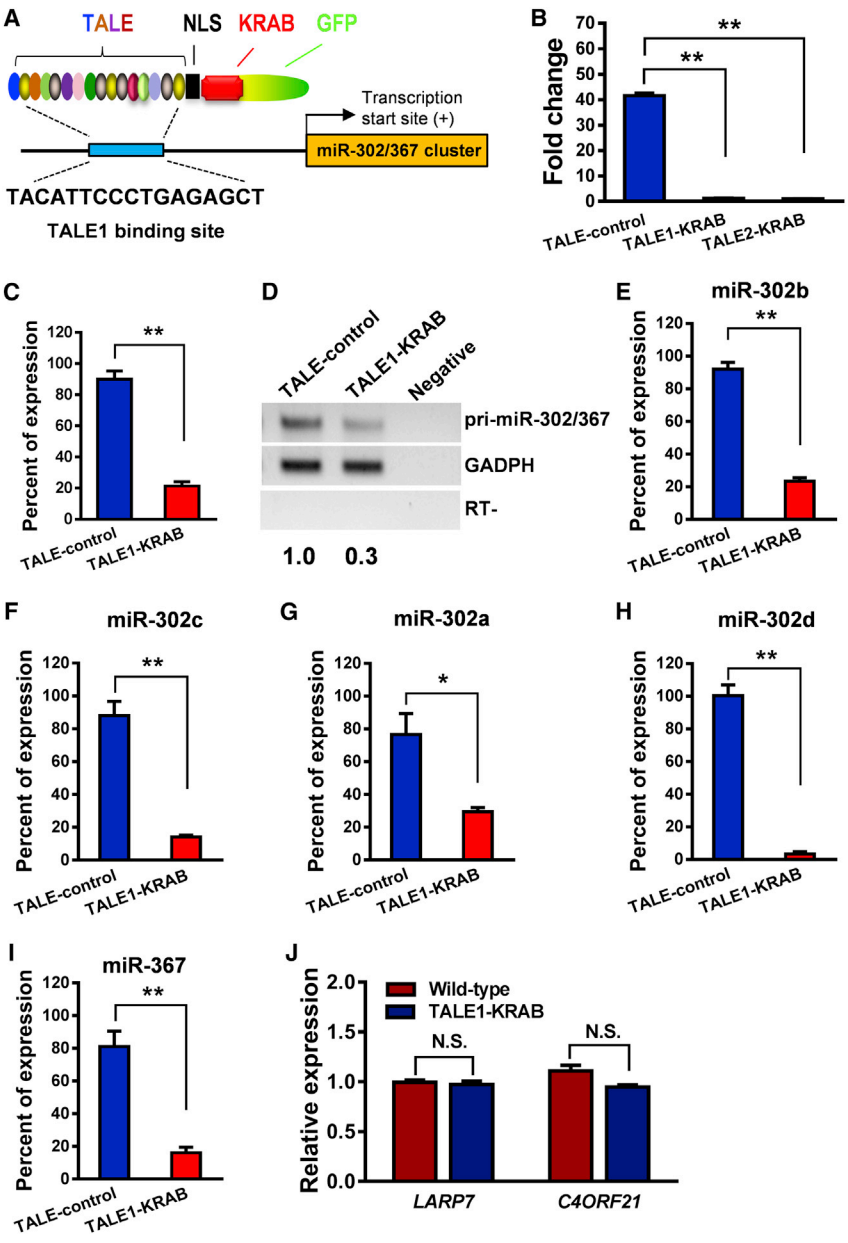

Figure 1. Knockdown of the miR-302/367 Cluster by TALE-KRAB Repressor Diminishes Reprogramming Efficiency

(A) Design of a miR-302/367 specific *TALE1-KRAB* transcriptional repressor for knockdown of the miR-302/367 cluster. A DNA-binding domain was designed to bind the indicated sequence within the proximal region upstream of the transcription start of the miR-302/367 cluster and fused to the N terminus of the KRAB transcriptional repressor domain, which is fused in-frame with GFP gene via 2A sequence. NLS, nuclear localization signal.

(B) Luciferase reporter assay of the miR-302/367 promoter-driven reporter (miR-302/367-Luc). 293T cells were transfected with miR-302/367-Luc and plasmids expressing TALE-control, TALE1-KRAB, or TALE2-KRAB, respectively. pCMV-LacZ was included in each transfection as an internal control to normalize luciferase activity. Data represent the mean ± SD (n = 3, independent transfection replicates). **p < 0.01, Student's t test.

(C) qPCR analysis of pri-miR-302/367 in hESCs expressing TALE1-KRAB. hESCs were infected with TALE-control or TALE1-KRAB. Transcripts of pri-miR-302/367 were analyzed by qPCR. Data are represented as the mean ± SD (n = 3, technical replicates). **p < 0.01, Student's t test.

(D) TALE1-KRAB inhibits expression of pri-miR-302/367 induced by OKSM reprogramming factors in HFFs. HFFs stably expressing OKSM were separately infected with TALE-control and TALE1-KRAB and then cultured for 1 week. Expression level of pri-miR-302/367 transcripts in transduced cells was analyzed by semiquantitative PCR. Relative density of DNA bands was quantified by ImageJ (NIH) and normalized to GADPH.

(E–I) qPCR analysis of mature miR-302b (E), miR-302c (F), miR-302a (G), miR-302d (H), and miR-367 (I) in HFFs stably expressing OKSM and TALE1-KRAB or TALE-control. Data represent the mean ± SD (n = 3, technical replicates). *p < 0.05, **p < 0.01, Student's t test.

(J) qPCR analysis of *LARP7* and *C4ORF21* transcripts. HFFs stably expressing OKSM were infected with lentiviruses-expressing TALE1-KRAB and cultured for 1 week. Total RNA was extracted from infected cells and analyzed for expression of *LARP7* and *C4ORF21* by qPCR analysis. Data represent the mean ± SD (n = 3, technical replicates). Groups were compared using Student's t test. NS, not significant.

such as miR-302/367, efficient approaches for knockout of miRNA or miRNA clusters in cell lines or primary cells are highly desired because it is extremely difficult, if not impossible, to efficiently knock out small regions encoding miRNAs by using traditional knockout approaches.

TALE technology provides an alternative approach. When TALE was fused with the Folk1 cleavage domain, it was shown to target endogenous genes and efficiently modify the genome (Hockemeyer et al., 2011). Thus, we decided to evaluate the effectiveness of TALENs for deleting the endogenous miR-302/367 locus and investigate the role of the miR-302/367 cluster in cellular reprogramming. We designed two pairs of TALENs targeting upstream and downstream of the miR-302/367 locus (Figure 4A) and

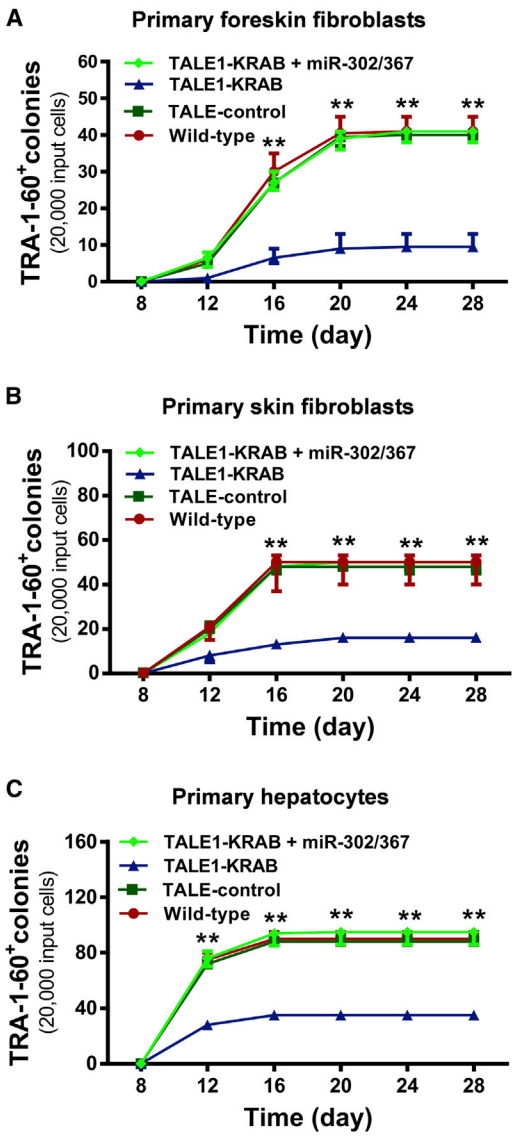

Figure 2. Effects of Knockdown of the miR-302/367 Cluster by TALE1-KRAB on Reprogramming Efficiency

(A–C) HFFs (A), primary skin fibroblasts (B), and primary hepatocytes (C) were transduced first with lentiviruses expressing TALE-control, TALE1-KRAB, or both TALE1-KRAB-expressing and miR-302/367 cluster-expressing viruses. These cells were then infected with OKSM-expressing retroviruses. Infected cells were seeded on feeder cells in six-well plates at a density of 20,000 transduced cells per well and cultured in hESC conventional medium for 4 weeks. Fully reprogrammed iPSC colonies were identified by immunofluorescence staining with anti-TRA-1-60 antibodies. Data are represented as the mean ± SD (n = 3, independent wells). The TALE-KRAB groups were compared with the other three groups using Student's t test. **p < 0.01.

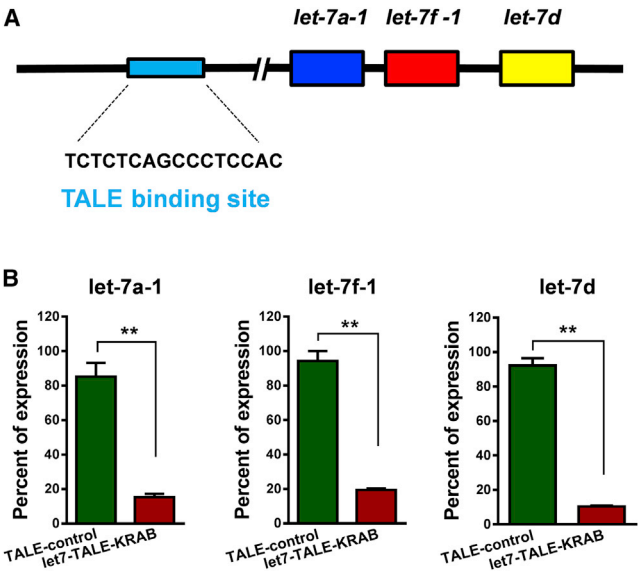

Figure 3. Design of TALE-KRAB for Knockdown of Human let-7a-1/let-7f-1/let-7d Cluster

(A) Diagram of human let-7a-1/let-7f-1/let-7d cluster and the target site for the designed TALE-KRAB. Human let-7a-1/let-7f-1/let-7d cluster consists of three miRNA members: let-7a-1, let-7f-1, and let-7d. The designed TALE-KRAB targeted a specific site as indicated in the promoter region of this cluster.

(B) qPCR analysis of let-7a-1, let-7f-1, and let-7d transcripts. 293T cells were transfected with TLAE-control or TALE-KRAB specific for the human let-7a-1/let-7f-1/let-7d cluster. Total RNA was extracted after 5 days of transfection and digested with DNase I to remove genomic DNA. A total of 500 ng total RNA was reverse transcribed into complementary DNA, and expression level of three members (let-7a-1, let-7f-1, and let-7d) was analyzed by qPCR. Data represent the mean ± SD (n = 3, technical replicates). **p < 0.01, Student's t test.

confirmed their cleavage activity in HFFs by PCR (Figure 4B). To replace the endogenous miR-302/367 cluster with a selection marker, we constructed a donor plasmid in which the CMV-eGFP-2A-Puro expression cassette was flanked with two arms that are homologous with the 5′ and 3′ regions of miR-302/367 cluster, respectively (Figure 4A). To determine the best ratio for the two pairs of TALEN expression constructs versus the donor plasmid, we cotransfected the donor vector with increasing amounts of TALEN expression plasmids into the HFFs and expanded the cells with medium containing puromycin for 1 week. We extracted genomic DNA from GFP$^+$ HFFs and used PCR to examine the site-specific recombination efficiency of the CMV-eGFP-2A-Puro expression cassette integrated into the miR-302/367 cluster. Our result indicated that the ratio of TALEN expression vectors to the donor plasmid affected the efficiency of knockout of the miR-302/367 cluster (Figure S1 available online). Notably, both alleles of the miR-302/367 cluster were completely deleted with high efficiency in the GFP$^+$ cells when equal amounts of the TALEN expression vectors and the donor

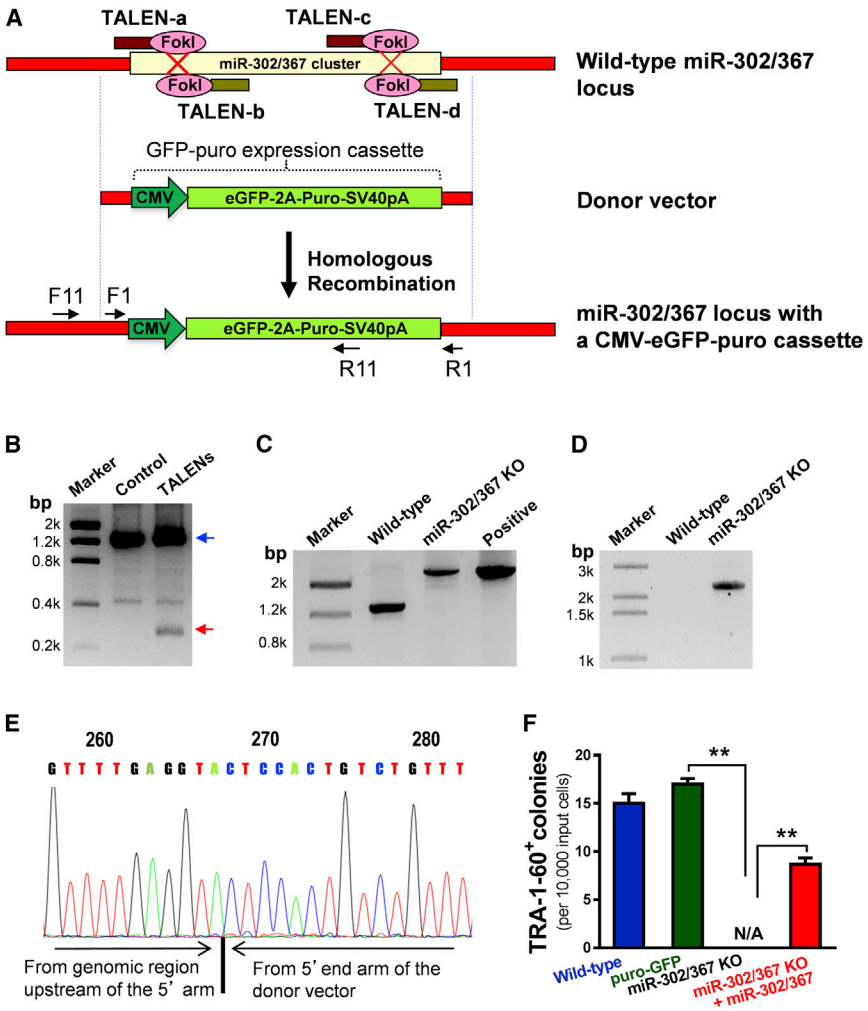

Figure 4. Deletion of the miR-302/367 cluster by TALENs Blocks Human iPSC Generation

(A) Diagram of strategy for TALENs-mediated knockout of the miR-302/367 cluster. Two pairs of TALENs (TALEN-a/b and TALEN-c/d) targeted their specific binding sites in the 5′ and 3′ ends of the miR-302/367 cluster, respectively. In the donor vector, the CMV-eGFP-2A-Puro expression cassette was flanked by the two homology arms (red font color) at the 5′ and 3′ ends, respectively. Homologous recombination (HR) was mediated by expression of the TALENs in the presence of the donor vector. Targeting leads to site-specific integration of the CMV-eGFP-2A-Puro expression cassette (green font color). Primers F1 and R1 were located in the 5′ and 3′ homology arms and near the CMV promoter and SV40pA, respectively. Forward primer F11 was located upstream of the 5′ arm and reverse primer R11 was located in GFP.

(B) Detection of cleavage activity of the TALENs designed for deleting the miR-302/367 cluster. Two pairs of the TALENs (TALEN-a/b and TALEN-c/d) were transfected into the HFFs, and the genomic DNA was extracted from transfected cells at 3 days following transfection. The DNA band (1.2 kb, blue arrow), corresponding to the regions covering miR-302/367 cluster, was amplified by PCR using primers F1 and R1 that were located outside the binding sites of the TALENs. An expected, 250 bp of DNA band (red arrow) was only amplified from cells transfected with the TALENs, and this DNA band indicates successful deletion of the miR-302/367 cluster.

(C and D) Site-specific integration of the CMV-eGFP-2A-Puro expression cassette from the donor vector. HFFs were transfected with equal amounts of donor plasmid and two pairs of TALEN expression vectors and then selected with puromycin at 48 hr after transfection. Genomic DNA was extracted from puromycin-resistant HFFs, and PCR was performed using primer pairs F1 and R1 (C) and F11 and R11 (D), respectively.

(E) Confirmation of correct site-specific integration of the CMV-eGFP-2A-Puro expression cassette. The DNA band shown in (D) was amplified using primers F11 and R11 and then confirmed by direct DNA sequencing.

(F) Effects of the miR-302/367 knockout on cellular reprogramming. HFFs were transfected with the donor vector and two pairs of the TALEN expression constructs, and then they were selected with puromycin for 1 week. After confirming knockout of the miR-302/367 cluster, the HFFs were transduced with viruses expressing OKSM (miR-302/367 KO group) or both miR-302/367-expressing and OKSM-expressing viruses (miR-302/367 KO + miR-302/367 group). Two control groups were included: one was wild-type HFFs, and the other one was HFFs transduced with pMig-puro-GFP retrovirus and selected with puromycin for 1 week (puro-GFP group). Cells in these two groups were finally infected with retroviruses expressing OKSM. For generating iPSCs, infected HFFs were seeded on feeder cells in 6-well plates at a density of 1×10^4 transduced cells per well and cultured in hESC culture medium for 3 weeks. For the miR-302/367 KO group, infected cells were seeded at a density of increasing numbers of transduced cells (1×10^4, 5×10^4, and 2×10^5) per well. True iPSC-like colonies were then confirmed by immunofluorescence staining using TRA-1-60 antibodies. Data represent the mean ± SD (n = 3, independent wells). **$p < 0.01$, Student's t test.

See also Figures S1–S4.

plasmid were transfected (Figure 4C). By PCR and DNA sequencing, we confirmed the correct site-specific integration of the CMV-eGFP-2A-Puro expression cassette into the miR-302/367 locus (Figures 4D and 4E). In addition, our qPCR analysis showed that the expression levels of the two genes *LARP7* and *C4ORF21* located near the miR-302/367 cluster were comparable in wild-type and miR-302/367 KO HFFs (Figure S2), indicating that knockout of the miR-302/367 did not change expression of the genes near this cluster. Together, these data indicate that use of TALENs is an effective and reliable approach for deleting miRNA clusters in cells.

Knockout of the miR-302/367 Cluster Completely Blocks iPSC Generation

Having HFFs deficient in the miR-302/367 cluster, we then asked a key question: Is the miR-302/367 cluster for iPSC generation? To address this question, we needed to generate and compare reprogramming efficiency of the four groups of HFFs: (1) wild-type HFFs, (2) HFFs overexpressing puromycin-GFP by retroviruses (puro-GFP), (3) HFFs deficient in the miR-302/367 cluster (miR-302/367 KO), and (4) HFFs deficient in the endogenous miR-302/367 cluster and overexpressing the exogenous miR-302/367 cluster (miR-302/367 KO + miR-302/367). Since senescence affects cellular reprogramming, we infected HFFs with different amounts of retroviruses expressing puromycin-GFP in a 6-well plate and cultured them in medium with puromycin for one week. The wells, which showed a similar cell density as the miR-302/367 KO group, were chosen as the control group (Figure 4F). Thus, HFFs in the control group (puro-GFP) and in the miR-302/367 KO group should undergo an equivalent degree of cell senescence during selection. We performed reprogramming assays by infecting the four groups of cells with retrovirus expressing four reprogramming factors OKSM. Our data showed that iPSC colonies emerged in the three groups of HFFs (groups 1, 2, and 4) after 3 weeks in our reprogramming culture condition. In contrast, HFFs null of the miR-302/367 cluster (group 3) did not form any iPSC colonies even with the reprogramming period extended from 3 to 8 weeks (Figure 4F). To ensure the reliability of our reprogramming, we validated some iPSC colonies by examining expression of several pluripotent markers in addition to AP and TRA-1-60 (Figures S3A and S3B). Together, our data indicated that reprogramming of HFFs deficient in the miR-302/367 cluster was dramatically impaired and was largely rescued by overexpression of an exogenous miR-302/367 cluster. These data suggested that the endogenous miR-302/367 cluster plays essential roles in cellular reprogramming.

Mesenchymal-to-epithelial transition (MET) plays important roles in cellular reprogramming (Li et al., 2010). It was recently reported that the miR-302/367 cluster promotes an increased expression of E-cadherin and accelerates MET change during reprogramming (Li et al., 2010, 2011; Liao et al., 2011). Thus, we assessed the expression of E-cadherin during the reprogramming process in both wild-type and miR-302/367 KO HFFs (Figure S4). Our qPCR analysis showed that knockout of the miR-302/367 cluster impaired the increased expression of E-cadherin at day 4 and 6 after reprogramming. These data suggest that the miR-302/367 cluster is essential for MET change during the early stage of reprogramming.

In conclusion, we demonstrate that TALE-KRAB and TALEN are very effective, reliable, and efficient loss-of-function approaches to study the roles of miRNA clusters in cellular reprogramming.

DISCUSSION

TALEs are natural effector proteins secreted by *Xanthomonas* and *Ralstonia* bacteria. These proteins regulate gene expression in host plants and thus facilitate bacterial survival and colonization (Moscou and Bogdanove, 2009). TALE contains a DNA-binding domain consisting of 34-amino-acid tandem repeat modules (Moscou and Bogdanove, 2009). Recently, significant efforts have been made toward genetic modification and transcriptional modulation by using TALEs (Cong et al., 2012; Hockemeyer et al., 2011) because designing TALE is much easier than zinc fingers and meganucleases. It was demonstrated previously that the KRAB repressor domain fused with TALE designed specifically to target the promoter of *SOX2* is able to inhibit the endogenous expression level of *SOX2* (Cong et al., 2012).

In this study, we used the KRAB repressor domain fused with a specific TALE1 targeting the promoter region of the miR-302/367 cluster to knockdown the expression level of this miRNA cluster during the reprogramming process. Our data showed that the endogenous expression of the miR-302/367 cluster was decreased 70%–90% more than the control group. Although miRZIP and LNA oligonucleotide inhibitors are relatively effective, it is very difficult to inhibit miRNA function with high specificity and efficiency simultaneously because specificity is inversely affected by the dosage of these inhibitors delivered into each cell. Additionally, LNA oligonucleotide inhibitors can only transiently inhibit miRNA function. Unlike miRZIP and LNA oligonucleotide inhibitors that restrain miRNA function by directly binding to target miRNA, TALE-KRAB repressors downregulate miRNA expression, leading to a reduced level of miRNA transcripts in cells. Therefore, TALE-KRAB repressors are promising and

powerful tools for knockdown of miRNA clusters because other knockdown approaches are not efficient in inhibiting mature miRNAs, making it impossible to perform simultaneous knockout of more than two miRNAs or entire miRNA clusters.

Loss-of-function studies of miRNAs by the knockout approach are in high demand as these molecules are important gene regulators in multiple biological processes. Conventional knockout strategies are not suitable for deleting miRNAs or miRNA clusters in primary cells or cell lines. Here, we knocked out the endogenous miR-302/367 cluster in HFFs by using specific TALENs and a donor vector. Notably, our data showed that almost all the HFFs were homozygous miR-302/367 null mutants after drug selection. We reasoned that the high efficiency of site-specific recombination of the GFP-puromycin expression cassette by the specific TALENs may be due to: i) the use of two, instead of one, pairs of TALENs, and ii) the use of a donor vector with puromycin selection marker. Interestingly, Ansai et al. recently reported that TALENs-induced mutation efficiency is mainly affected by TALENs activity, target sequences, and the amount of TALENs transfected or injected in each cell. They showed that a high concentration of TALENs induced extremely high mutation efficiency (almost 100%), and all the mutants were homozygous (Ansai et al., 2013).

In summary, we demonstrate here that TALE-based transcriptional repressors and TALENs are two promising loss-of-function approaches for dissection of functional roles of miRNAs or miRNA clusters during cellular reprogramming or in other biological processes.

EXPERIMENTAL PROCEDURES

Generation of Human iPSCs

HFFs were seeded in a 6-well plate at 1×10^4 cells per well 1 day before transduction and incubated with a virus-containing supernatant supplemented with 5 μg/ml polybrene (Sigma), followed by centrifugation at 1,000 × *g* for 30 min. Four days postinfection, cells were split using 0.025% trypsin-EDTA and plated on mouse embryonic fibroblast feeders cultured in fibroblast medium. After 24 hr, medium was switched to the conventional hESC medium plus 0.5 mM sodium butyrate (Sigma). Media were changed every other day until induced colonies were picked up based on hESC colony morphology at day 21. iPSC colonies were stained by alkaline phosphatase in situ and antibodies against TRA-1-60, TRA-1-81, OCT4, SOX2, or SSEA4.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, one table, and six DNA sequences for the *TALE-KRAB* and *TALENs* and can be found with this article online at http://dx.doi.org/10.1016/j.stemcr.2013.07.002.

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