Imprinted gene *Zinc finger protein 127* is a novel regulator of master pluripotency transcription factor, Oct4

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Induced pluripotent stem cells (iPSCs) show great promise for replacing current stem cell therapies in the field of regenerative medicine. However, the original method for cellular reprogramming, involving four exogenous transcription factors, is characterized by low efficiency. Here, we focused on using epigenetic modifications to enhance the reprogramming efficiency. We hypothesized that there would be a new reprogramming factor involved in DNA demethylation, acting on the promoters of pluripotency-related genes. We screened proteins that bind to the methylated promoter of *Oct4* and identified Zinc finger protein 127 (Zfp127), the functions of which have not yet been identified. We found that Zfp127 binds to the *Oct4* promoter. Overexpression of Zfp127 in fibroblasts induced demethylation of the *Oct4* promoter, thus enhancing *Oct4* promoter activity and gene expression. These results demonstrate that Zfp127 is a novel regulator of *Oct4*, and may become a potent target to improve cellular reprogramming. [BMB Reports 2018; 51(5): 242-248]

INTRODUCTION

One of the major advantages of induced pluripotent stem cells (iPSCs) is their potential application in cell and regenerative therapies, and thus there have been numerous attempts to generate them. In 2006, a mouse fibroblast was successfully reprogrammed with four transcription factors, Oct4, Sox2, Klf4, and c-Myc (1). These transcription factors were ectopically overexpressed with viral transduction. Despite the many advantages that iPSCs can offer for cell therapy, low efficiency and tumorigenicity still remain major issues impeding their clinical applications. Therefore, many researchers have endeavored to improve their production efficiency, and to reduce the tumorigenic risks. Consequently, numerous experimental methods for generating iPSCs using plasmids, adenoviral transduction, miRNA, and protein extracts have been developed recently (2-7). Among them, several studies have focused on discovering new reprogramming factors (8-11).

It is widely known that gene expression patterns change with the epigenetic modifications made during reprogramming (12-15). The DNA of the promoters of pluripotency-related genes such as *Oct4* and *Nanog* can change between methylated and unmethylated states (16-18). Oct4 mRNA is highly expressed in iPS cells, compared with somatic cells, which exhibit very low level expression. The differences in Oct4 expression originate from the methylation status of the *Oct4* promoter. Therefore, we focused on the differences in the methylation patterns of the *Oct4* promoter, and attempted to identify a novel reprogramming factor associated with the demethylation of the *Oct4* promoter.

Based on our previous studies (6, 19), where we generated iPSCs by exposing fibroblasts to cellular extract of embryonic stem cells or iPSCs, we hypothesized that the extract of pluripotent stem cells may contain reprogramming factors that bind to the methylated *Oct4* promoter, and demethylate it during reprogramming. We aimed to discover these reprogramming factors by screening proteins bound to the methylated *Oct4* promoter, and as a result, identified Zinc finger protein 127 (Zfp127). Although the function of Zfp127 has not been reported, its amino-acid sequence includes a zinc finger domain, leading us to predict that Zfp127 is capable of binding both DNA and RNA with high affinity. The *Zfp127* gene is maternally imprinted, and located on mouse chromosome 7 (19).

Here, we have identified a novel function of Zfp127. We report that Zfp127 binds to the *Oct4* promoter and

demethylates it, leading to an increased expression of the *Oct4* gene. We therefore speculate that Zfp127 influences the reprogramming process by positively regulating Oct4, the key transcription factor involved in reprogramming.

RESULTS

The *Zfp127* gene was identified after screening proteins that bind to the methylated *Oct4* promoter

CpG islands in the *Oct4* promoter region are demethylated during cellular reprogramming, as shown in many previous studies (1, 2, 6, 20). Expression of Oct4 is largely regulated by the methylation status of the promoter. Therefore, we focused on finding the factor which binds to the methylated *Oct4* promoter and modifies its methylation status. Methylation patterns on the *Oct4* promoter show a distinct contrast between pluripotent stem cells and somatic cells (21, 22). We used the *Oct4* promoter region, from −466 to +22 bp, relative to the transcription start site, in our experiments (Fig. 1A).

The *Oct4* promoter was methylated artificially by CpG methyltransferase. Its methylation state was analyzed by digestion with MspI and HpaII. MspI recognizes the sequence, CCGG, digesting the *Oct4* promoter into fragments of 266, 221, and 46 base pairs. HpaII is an isoschizomer of MspI, and is prevented from digesting at CCGG by the presence of a 5-methyl group at the internal C residue of its recognition sequence (Fig. 1B). Therefore, MspI digested the methylated *Oct4* promoter, but HpaII did not digest (Fig. 1C). Next, the methylated *Oct4* promoter was incubated with protein extracts from mESC. Based on our previous studies where we generated iPSC by treating fibroblasts with mESC extracts, we hypothesized that the mESC extracts contain a reprogramming-related factor that binds to the *Oct4* promoter (6, 7). To evaluate whether proteins were bound at Oct4 promoter with distinguishing methylation state, we performed western blot analysis with Oct4 and MBD2 antibodies after DNA (unmethylated or methylated Oct4 promoter) and proteins (mESC extract) were mixed together, then incubated and washed to remove unbound proteins. This process was described in Supplementary Fig. S1A as schematic figure. Oct4 protein was detected only at mixture of unmethylated DNA and mESC extract but MBD2 was detected at mixture of methylated DNA and mESC extract (Supplementary Fig. S1B). This is consistent with previous report (23). However, Lamin A/C, which contains no DNA binding domain, was detected neither mixture of methylated or unmethylated DNA and mESC extracts (data not shown). This data demonstrated that our screening system is valuable to find novel methylated DNA binding proteins. To discover methylated DNA binding proteins, DNA (methylated *Oct4* promoter) and proteins (mESC extract) were mixed together, then incubated, washed and eluted the proteins, which were then separated by gel electrophoresis, purified, and analyzed by MALDI-TOF. We identified Zfp127 through this MALDI-TOF (Fig. 1D). We also
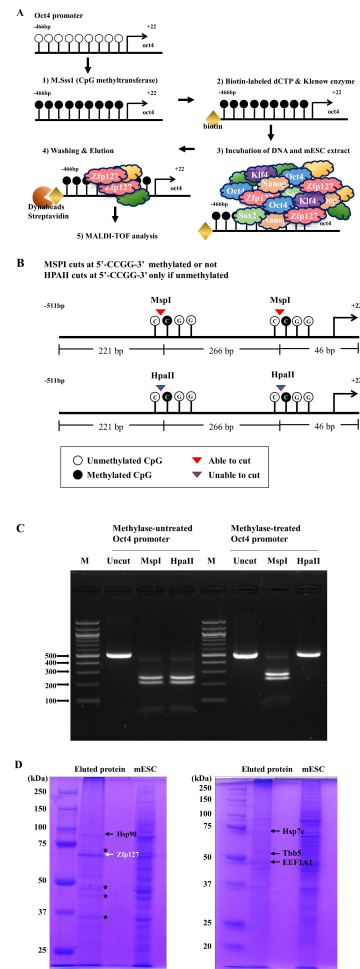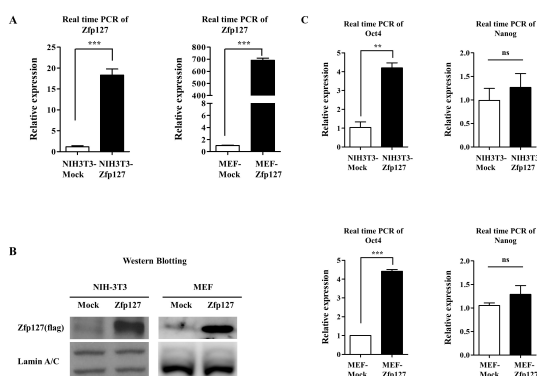

Fig. 1. Schematic diagram to screen proteins from the mESC extract that bind to the methylated *Oct4* promoter. (A) *Oct4* promoter (sequence from −466 to +22 bp) was selected to screen the proteins from the mESC lysate that binds to the methylated *Oct4* promoter. Artificially methylated *Oct4* promoter was incubated with mESC extract. The proteins that bind to the methylated *Oct4* promoter were eluted and separated using SDS-PAGE gel electrophoresis. They were then analyzed with MALDI-TOF. (B, C) The methylation state of the *Oct4* promoter was confirmed by digestion with Msp I and Hpa II. (D) Eluted proteins included Hsp90, Hsp7c, Tbb5, EEF1A1 and Zfp127. *(asterisk) indicate unknown proteins.

identified Hsp90, Hsp7c, Tbb5, EEF1A1 through this analysis (Fig. 1D and Supplementary Table 1). Since the sequence of *Zfp127* includes zinc finger domains, it can be translated into a putative protein with DNA or RNA binding ability. However, other proteins contain no DNA binding domain and are located at cytoplasm. Therefore, we speculated that only Zfp127 is likely to be a protein that binds to the methylated DNA.

Zfp127 increases Oct4 expression by inducing demethylation of the *Oct4* promoter

To investigate whether Zfp127 affects the gene expression of *Oct4*, we constructed a vector to overexpress the *Zfp127* gene in the NIH-3T3 cell line and mouse embryonic fibroblasts (MEFs). The overexpression of *Zfp127* was confirmed by mRNA and protein levels, using real-time PCR and western blot analysis respectively (Fig. 2A, B). Real-time PCR showed upregulated Oct4 mRNA expressions when Zfp127 was overexpressed. However, there was no change in gene expression levels of *Nanog* in the Zfp127 over-expressing cells (Fig. 2C). Next, we examined whether Zfp127 regulates the methylation state of the *Oct4* promoter. After Zfp127 was overexpressed in NIH3T3 cells, we analyzed the methylation status of the *Oct4* promoter using bisulfite sequencing. There are 16 CpG islands in the *Oct4* promoter region from -466 to $+20$ bp. Among them, CpG islands located between $+21$ and -244 bp of the *Oct4* promoter region were methylated, while the -268 to -466 bp region was completely unmethylated (Fig. 3A). The *Oct4* promoter ($+21$ to -244 bp) region in the NIH-3T3 cell line had been reported as being unmethylated, which was thought to be a characteristic of this cell-line (24). Thus, we conducted bisulfite sequencing analysis in order to individually examine 10 CpG islands within the promoter ($+21$ to -244 bp) region of mock and *Zfp127*-transfected NIH3T3 cells. The frequency of the unmethylated CpG islands in *Zfp127*-transfected cells was twice as high as that of mock-transfected cells (Fig. 3B). The demethylation of the *Oct4* promoter can account for the increased Oct4 expression. This suggests that Zfp127 plays a role in demethylation of the *Oct4* promoter and *Oct4* gene expression, and may also facilitate cellular reprogramming. To rule out the possibility that Zfp127 can induce non-specific
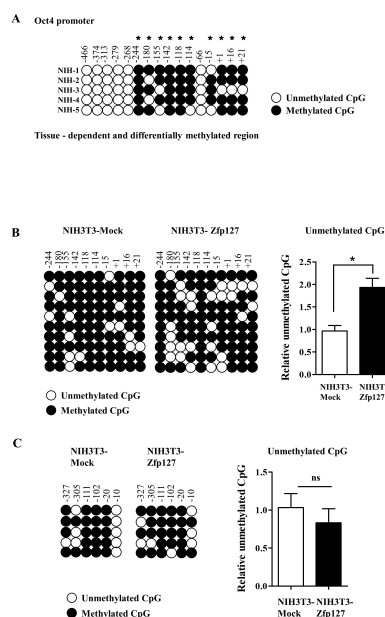
demethylation of DNA, we analyzed the expression level of *Nanog*, and the methylation status of its promoter in Zfp127-overexpressing cells. Unlike Oct4, Zfp127 overexpression did not affect the gene expression of *Nanog* (Fig. 2C), nor the methylation status of the *Nanog* promoter (Fig. 3C).

Zfp127 binds to the *Oct4* promoter and positively regulates its activity

Next, we investigated whether the demethylation of the *Oct4* promoter is linked to its activation. We constructed a luciferase vector containing a proximal enhancer and a promoter of *Oct4* (-466 to $+1$ bp) (Fig. 4A), which was then co-transfected with the Zfp127 overexpression vector in NIH3T3 and MEFs. As shown in Fig. 4B, Zfp127 overexpression increased *Oct4* promoter activity in NIH3T3 and MEFs by 1.7 and 2-fold greater, respectively, than the mock-control did. Next, to investigate whether Zfp127 binds and activates the *Oct4* promoter, we performed a ChIP assay. The Zfp127 overexpression vector includes a FLAG tag, and thus we performed our ChIP assay with an anti-FLAG antibody. We confirmed that Zfp127 binds to the *Oct4* promoter (Fig. 4C). This result demonstrates that the binding of Zfp127 increases the activity of *Oct4* promoter. Therefore, we can postulate that

Fig. 2. Overexpression of Zfp127 increases *Oct4* mRNA expression. (A, B) Real-time PCR and western blot results showed that high levels of *Zfp127* were detected in Zfp127-overexpressing NIH3T3 cells and MEFs. (C) Overexpression of Zfp127 increased *Oct4* expression, but not the expression of *Nanog* to significant levels. The asterisks indicate statistically significant changes: **P ≤ 0.01, ***P ≤ 0.001.

Fig. 3. DNA methylation analysis of *Oct4* and *Nanog* promoters in Zfp127 overexpressing fibroblasts. (A) Methylation patterns of the CpG islands of the *Oct4* promoter region from -466 to $+21$ bp in normal NIH-3T3 cells. Only 10 CpG islands are methylated in this region. (B) Bisulfite sequencing analysis of 10 CpG islands of the *Oct4* promoter. Partial demethylation of the *Oct4* promoter was observed in the Zfp127-overexpressing NIH-3T3 cells. (C) Demethylation of the *Nanog* promoter was not observed in the Zfp127 overexpressing NIH-3T3 cells. The asterisks indicate statistically significant changes: *P ≤ 0.05.
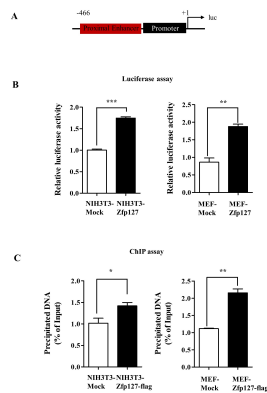
Fig. 4. Zfp127 activates and binds to the *Oct4* promoter in luciferase assay. (A) The *Oct4* promoter was cloned into the pGL2 luciferase vector. (B) Activation of the *Oct4* promoter increased in Zfp127-overexpressing NIH-3T3 cells and MEFs. (C) Quantification of the ChIP assay by real-time PCR with primers specific for the *Oct4* promoter. The results are normalized to input. Binding of Zfp127 onto the *Oct4* promoter increased in Zfp127 overexpressing NIH-3T3 cells and MEFs. The asterisks indicate statistically significant changes: *P ≤ 0.05, ***P ≤ 0.001.

the induction of the *Oct4* gene expression was due to the binding of Zfp127 on the *Oct4* promoter, resulting in its demethylation. To confirm the transcriptional activity of *Oct4*, we investigated the mRNA levels of the well-known target genes of Oct4. Since *Sox2* and *Foxd3* are early response genes of the Oct4 transcription factor (25), we choose them as downstream targets of Oct4. Real-time PCR showed upregulated mRNA levels of these genes when Zfp127 was overexpressed (Supplementary Fig. S2A). Combined, these results suggest that *Oct4* is not only a direct target of Zfp127, but also has transcriptional activity.

DISCUSSION

Reprogramming somatic cells into pluripotent stem cells can be employed in various basic and clinical applications. Using iPSCs does not lead to problems such as ethical limitations and immune rejection after organ transplant. Despite many advantages of iPSCs in cell therapy, the low efficiency and time-consuming process of iPSC generation, as well as its tumorigenic risks, are significant handicaps for clinical applications. Through optimization of the reprogramming methods, these problems can be avoided (8-11, 26).

Our study aimed to find a new reprogramming-related protein, and to determine a novel function of this molecule, to contribute to the technological advancements in iPSC generation methods. Here, we designed a new experiment to screen reprogramming-related factors. Epigenetic changes occur when somatic cells are reprogrammed. One of the important epigenetic modifications which takes place during reprogramming is the removal of DNA methylation on the

promoters of pluripotency-regulated genes (16, 21, 22). We assumed that there would be some unknown reprogramming factors playing important roles in demethylating the *Oct4* promoter. We hypothesized that mESCs bear these factors. This assumption is based on our previous study, for which we succeeded in making iPSCs by treating fibroblasts with protein extracts of mESCs (6, 7).

To screen for this DNA demethylase, we mixed and incubated the methylated *Oct4* promoter with mESC extract, and analyzed the proteins that bound to the promoter, using MALDI-TOF (Fig. 1A). The methylation status of the *Oct4* promoter is critical in regulation of Oct4 expression, as indicated by the different expression patterns seen in somatic and pluripotent cells (18, 21, 22). For this reason, we chose to use the *Oct4* promoter for our experiment. The analysis of our MALDI-TOF data allowed us to identify a complex of several compounds that bound to the methylated *Oct4* promoter. The bands we obtained by SDS PAGE were a mixture of several different proteins, from which we were able to identify Hsp90, Hsp7c, Tbb5, EEF1A1, and Zfp127, as shown in the results (Fig. 1D and Supplementary Table 1).

Zfp127 is a maternally imprinted gene, expressed only by the paternal allele, and is translated into a putative protein possessing zinc finger motifs (19, 27). This imprinted region is homologous to human locus 15q11-q13, where its imprinting mutation results in Prader-Willi syndrome (PWS) (19). The mouse *Zfp127* and the human orthologue show similar characteristics. They are expressed only by paternal alleles, and their sequences encode conserved proteins with five putative zinc finger motifs. These motifs imply that this protein may play important roles in DNA or RNA binding. The *in vitro* translation of human *Zfp127* showed that it is likely to be translated *in vivo* and may encode a functional protein (27). This suggests that mouse *Zfp127* may also encode a functional protein, and it is likely to regulate gene expression through its zinc finger motifs.

Although several proteins were obtained during the experiment, Zfp127 was selected as a feasible reprogramming factor based on its zinc finger motifs. The function of the *Zfp127* gene remains to be unveiled. Hence, we investigated the effects of the *Zfp127* gene on the *Oct4* promoter. As mentioned above, Zfp127 may affect the *Oct4* promoter since expression of *Oct4* mRNA increased in Zfp127 overexpressing cells. For this reason, we postulated that it may be due to the effects of Zfp127 on the *Oct4* promoter.

We examined the methylation status of the *Oct4* promoter and found that methylation of some CpGs were removed in the Zfp127 overexpressing cells. Next, to find out whether Zfp127 can demethylate other regions of the promoters of pluripotent factors nonspecifically, we checked the expression of the *Nanog* gene and its promoter methylation status, as Nanog is also a key transcription factor involved in reprogramming. However, neither the removal of the CpG methylations, nor the change in gene expression of *Nanog*

were observed. Next, we confirmed the activation of the *Oct4* promoter with a luciferase assay. Our analysis of the *Oct4* promoter using bisulfite sequencing and a luciferase assay leads us to believe that the activation of the *Oct4* promoter might be due to its demethylation.

In this study, we have demonstrated that Zfp127 induces Oct4 expression by demethylation of the *Oct4* promoter specifically. However, Zfp127 contains no demethylase domain. Whether other DNA demethylating proteins are recruited to the proximal region, followed by Zfp127, remains to be investigated. The fact that the Zfp127 protein possesses zinc finger motifs and induces demethylation of CpG islands suggests that Zfp127 may have the ability to bind to the promoters of reprogramming-related genes other than *Nanog*. It is possible that there may be some other factors that interact with Zfp127, which influence the specificity of the binding sites.

To identify proteins that have DNA demethylase domains and interact with Zfp127 in mESC extracts, we performed a proteomic analysis using iTRAQ and Mass/Mass experiment. We compared pull-downs of mESC proteins, which were bound to either unmethylated or methylated *Oct4* promoters respectively. However, the analysis did not identify any known DNA demethylation-related proteins, such as DNA deaminase (18) or methylcytosine oxidases (28). This suggests that there are unknown DNA demethylases which interact with Zfp 127 to demethylate the *Oct4* promoter.

The finding that Zfp127 increases Oct4 expression, may be useful for practical applications, such as facilitating cellular reprogramming and improving the efficiency of generating iPSCs, offering the possibility of Zfp127 as a new reprogramming factor. The combination of Zfp127 and other known transcription factors can be introduced to somatic cells to enhance reprogramming efficiency.

In summary, we have identified a novel function of an imprinting gene, *Zfp127*. It binds to the *Oct4* promoter, leading to demethylation of the *Oct4* promoter. The promoter of *Oct4* was then activated, and the expression of *Oct4* mRNA increased. Therefore, we speculate that Zfp127 offers the potential for somatic cells to be reprogrammed into iPSCs with high efficiency.

MATERIALS AND METHODS

Cell culture
Mouse embryonic stem cells were derived from C57BL/6-background mouse embryonic stem cells (American Type Culture Collection). mESCs were cultured as described in previously (6, 20). Briefly, one day before sub-culturing mESCs, mitomycin C (10 μg/ml medium (M4287)(Sigma-Aldrich, USA))-treated STO cells were seeded on a new 0.1% gelatin-coated dish. The STO cells were cultured in Dulbecco's modified Eagle's medium (DMEM)(11995-065) (Invitrogen, USA) high glucose, supplemented with 10% Fetal

Bovine Serum (FBS)(16000-044)(Invitrogen, USA), and 100 U/ml penicillin streptomycin (Gibco). mESC were cultured in (DMEM)(11995-065)(Invitrogen, USA) with 10% FBS (FBS)(16000-044)(Invitrogen, USA), 2 mM L-glutamine (25030-081)(Invitrogen, USA), 1X NEAA (Gibco) (11140-050) (Invitrogen, USA), 1 mM 2- mercaptoethanol (Sigma-Aldrich), and 100 U/ml penicillin/100 μg/ml streptomycin (Gibco) (15140-122)(Invitrogen, USA)(ES media). In ES media, 2,000 U/ml of ESGRO® LIF (leukemia inhibitory factor; (ESG1107) (Chemicon, USA)) was added to maintain pluripotency. mESC were dissociated with 0.05% trypsin (Gibco)(25300-054) (Invitrogen, USA), and passaged on STO every 2-3 days. Cells from passages 5 to 7 were used for further experiments.

The NIH-3T3 cell line and the mouse embryonic fibroblasts were purchased from the American Type Culture Collection. NIH-3T3 and MEFs were incubated at 37°C in Dulbecco's modified Eagle's medium (DMEM)(11995-065)(Invitrogen, USA), high glucose, supplemented containing 10% Fetal Bovine Serum (FBS)(16000-044)(Invitrogen, USA), and 100 U/ml penicillin streptomycin (Gibco)(15140-122)(Invitrogen, USA).

Extraction of mESC protein
mESCs were washed in PBS and harvested with 0.1 mM trypsin/1 mM EDTA solution, as described previously (6, 29). Cells were collected by centrifugation (200 g for 10 min at 4°C) and resuspended in 1 ml cold cell lysis buffer (100 mM HEPES, pH 8.2, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, and protease inhibitor cocktail (04 693 132 001)(Roche, Switzerland). Cells were mixed by vortexing on ice for 1 h, and homogenized using a 20-gauge needle. The lysate was centrifuged at 15,000 rpm (22,250 g) for 30 min at 4°C, and the supernatant was stored at −80°C. The protein concentration was measured with a BCA protein assay kit (23255)(Thermo Scientific, USA).

In vitro methylation assay
An amplified *Oct4* promoter product (500 ng) was methylated by M.SssI methylase (M0226S)(New England Biolabs, UK), at 37°C for 3 h, with the subsequent inactivation of enzyme at 60°C for 20 min. To identify of the methylation state of *Oct4* promoter, the methylated product was digested with Msp I (R057S)(Enzynomics, Korea) and Hpa II (R049S)(Enzynomics, Korea).

Screening for the methylated *Oct4* promoter binding protein
A vector containing mouse *Oct4* promoter was constructed. The DNA vector was treated with the appropriate restriction enzyme, and only the mouse *Oct4* promoter was purified. The purified DNA was methylated artificially with CpG Methyltransferase (M.SssI;(M0226S)(New England Biolabs, UK)), and biotinylated with biotin-labeled dCTP and Klenow enzyme. Biotinylated DNA was attached to streptavidin with Dynabeads kilobaseBINDER kit (60101)(Invitrogen, USA). The

DNA was then incubated with the mESC protein extract. To remove the unbound proteins, beads were washed with washing buffer and magnets. Magnets pull streptavidin-Dynabeads, which were attached to biotinylated DNA with bound proteins. The last step was the elution of the proteins from the DNA and the protein analysis. After treatment with DNase (M6101)(Promega, USA) to eliminate DNA, we carefully took the supernatant. Finally, eluted proteins were separated using SDS-PAGE gel electrophoresis, and analyzed with MALDI-TOF and western blotting with Oct4 (sc-5279)(Santa Cruz, USA) and MBD2 (ab38646) (Abcam, UK) antibodies.

Western blot analysis

Cells were harvested and lysed in lysis buffer containing protease inhibitor cocktail (04 693 132 001)(Roche, Switzerland). Total protein (20-30 mg) was immunoblotted with primary antibodies against FLAG (F7425)(Sigma Aldrich, USA). Lamin A/C (2032S)(Cell Signaling Technology, USA), was used as an internal control.

Plasmid construction and transfection

The *Zfp127* gene was amplified with 2x Pfu premix (Solgent). RNA of mESC was used as a template. The amplified DNA was ligated into the T-Blunt vector using a T-Blunt PCR Cloning Kit (SOT02-K020)(Solgent, Korea). The *Zfp127* gene was sub-cloned into FLAG tagged pCMV-Tag 2B (FLAG-Zfp127). The whole sequence of *Zfp127* was confirmed through DNA sequencing. To overexpress Zfp127, FLAG-Zfp127 was transfected to NIH-3T3 cells by using of polyethyleneimine (PEI (23966)(polyscience, Inc. USA)) and was transfected to MEFs, by using the Amaxa nucleofection system (Lonza, Switzerland).

RNA preparation and PCR analysis

The total RNA of each cell line was purified with RNeasy Mini Kit (74104)(Qiagen, Germany), and cDNA was synthesized with an amfiRivert cDNA synthesis premix (Gendepot). The conventional RT-PCR was performed with a Maxime PCR Premix (25265)(Intron Biotechnology, Korea), and quantitative real-time PCR was performed with SYBR Green master mix (04913914001)(Roche, Switzerland). The following primer sets were used: 5'-AAGCGTGCAGGTATTTTGCT-3' and 5'-ACTGCACAGGC TCCAAAACT-3' for the *Zfp 127* gene; 5'-GAGGAGTCCCAG GACATGAA-3' and 5'-AGATGGTGGTCT GGCTGAAC-3' for the *Oct4* gene.

Genomic DNA preparation and bisulfite sequencing analysis

Genomic DNA of each cell line was extracted with a Genomic DNA extraction kit (17045)(Intron Biotechnology, korea). Conversion of the genomic DNA was performed with an EpiTect Bisulfite kit (59104)(Qiagen, Germany). The converted DNA was used as a template to amplify the *Oct4* promoter region. Amplified DNA was then purified and ligated to the pGEM-T easy vector (A1360)(Promega, USA) for sequencing. The primer sets used for the bisulfite sequencing are as follows:

5'-TGGGTTGAAATATTGGGTTTATTT-3'and 5'-CTAAA AC CAAATATCCAACCATA-3' for the *Oct4* gene, 5'-GATTTGTAG GTGGGATTAATTGTGAATTT-3'and 5'-ACCAAAAA AACCCA CACTC ATATCAATATA-3' for the *Nanog* gene (30).

Luciferase assay

To study *Oct4* promoter activity, cells were transfected with *pGL2-Oct4* promoter, luciferase reporter vector, and Renilla vector using polyethyleneimine (PEI)(23966)(polyscience, Inc. USA), approximately 1 or 2 days before detection. Cells were lysed with lysis buffer, and luciferase activity was detected with Dual-Luciferase Reporter Assay System (E1910)(Promega, USA).

Chromatin immunoprecipitation assay

A chromatin immunoprecipitation (ChIP) assay was performed as described previously (19, 28). In brief, Zfp127 overexpression was performed in NIH-3T3 and MEFs as mentioned above. 1×10^6 cells of Zfp127-overexpressing NIH-3T3 and MEFs were rinsed and treated with 1% formaldehyde for 20 min at room temperature. Samples were then sonicated on ice and incubated with the antibodies; anti-FLAG (sc-807)(Santa Cruz, USA) and anti-IgG antibody at $4^{\circ}C$ overnight. Immunoprecipitated DNA was analyzed using real-time PCR. The following primers were used to amplify the primer of the *Oct4* promoter; forward,

5'-GGAGGTGCAATGGCTGTCTTGTCC-3', and reverse, 5'-CTGCCTTGGGTCACCTTACACCTCAC-3'.

Statistical analysis

The results are expressed as means $\pm$ standard deviations (SD). The differences between the groups were compared using an unpaired t-test, followed by post-hoc analysis with the Bonferroni test, and P values $\leq$ 0.05 were considered statistically significant. All statistical analyses were performed using SPSS 17.0 (SPSS Inc., USA).

CONFLICTS OF INTEREST

The authors have no conflicting interests.

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