Development of programmable small DNA-binding molecules with epigenetic activity for induction of core pluripotency genes

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ABSTRACT

Epigenetic modifications that govern the gene expression are often overlooked with the design of artificial genetic switches. N-*Methylpyrrole*-N-*methylimidazole (PI) hairpin polyamides are programmable small DNA binding molecules that have been studied in the context of gene regulation. Recently, we synthesized a library of compounds by conjugating PI polyamides with SAHA, a chromatin-modifier. Among these novel compounds, PI polyamide–SAHA conjugate **1** was shown to epigenetically activate pluripotency genes in mouse embryonic fibroblats. Here, we report the synthesis of the derivatives of conjugate **1** and demonstrate that these epigenetically active molecules could be developed to improve the induction of pluripotency factors.*

1. Introduction

Genomic DNA is the decisive blueprint of our heredity and carries an immense corpus of information[1]. Collection and recovery of the information pertaining to the lineage commitment of the cell is controlled by the transcriptional machinery[2]. Misregulation of transcription is associated with at least 50% of tumors[3]. Therefore, the development of artificial transcriptional activators that can restore and regulate the expression of perturbed genes is undergoing vigorous study owing to their versatile medicinal applications[4–6]. Factors that can modulate the nucleobases of DNA will also have a significant impact on the cell fate. Consequently, naturally occurring DNA-binding proteins have been investigated for their medicinal applications[7]. Programmable natural transcriptional activators, such as designer polydactyl zinc fingers, homing endonucleases, and transcription activator-like effectors, have offered a viable way of achieving sequence-specific DNA perturbation[8–10].

Ever since the elucidation of the genetic code, it has been clear that genome information alone is insufficient to control gene expression, because humans and *Drosophila* have almost the same numbers of gene families[11]. In nature, gene expression is precisely regulated at the epigenetic level, and this predominantly involves the modification of histone proteins, which are suggested to have a code of their own, the histone code[12]. However, none of the known transcriptional activators can be developed as a genetic switch because they lack the consideration of the most critical epigenetic constraints[13].

The artificial transcriptional activation of pluripotency in somatic cells to generate induced pluripotent stem cells (iPSCs) has brought us a step closer to customized patient-specific cell therapies[14–16]. However, although promising, iPSC techniques have several shortcomings, such as the potential of retroviruses to cause tumors in tissues derived from host iPSCs, the low efficiency of induction, the limited duration of the reprogramming process, and the need for drug-resistance-based selection[17]. Despite recent promising breakthroughs, the clinical translation of iPSCs is still hindered by various phenomena, including the retention of epigenetic memory[18,19]. Epigenetic modifiers, including DNA methyltransferase (DNMT) inhibitors and/or histone deacetylase (HDAC) inhibitors, have been shown to enhance reprogramming efficiency[20–22]. However, the aforementioned chromatin modifiers lack selectivity, and because chromatin modification can induce heritable cell states, their precise application is essential for the safe clinical use of iPSCs.

Recently, we have developed artificial transcriptional activators that include sequence-specific hairpin pyrrole–imidazole (PI) polyamides conjugated with chromatin-modifying suberoylanilide hydroxamic acid (SAHA)[23]. Unlike other programmable DNA binding molecules, PI polyamide–SAHA conjugates are also epigenetically active[24]. Mouse embryonic fibroblast (MEF) cells were treated individually with a total of 16 PI polyamide–SAHA conjugates, and the effects of the conjugates on the expression of iPSC factors were screened. Our results indicated that PI polyamide–SAHA conjugate **1** (Fig. 1) increased the expression of *Oct-3/4* and *Nanog* by about three-fold. About 1.5–2-fold increase were also observed in the expression of *Sox2*, *Klf4*, and *c-Myc*[24]. In this paper, the design and synthesis of derivatives of the successful PI polyamide–SAHA conjugate **1** are reported, together with their effects on the expression patterns of five iPSC factors. Our results indicate that these programmable small DNA-binding molecules, which induce the epigenetic activation of specific gene(s), can be developed to induce the specific expression of core pluripotency genes that regulates the transcriptional machinery for pluripotency.

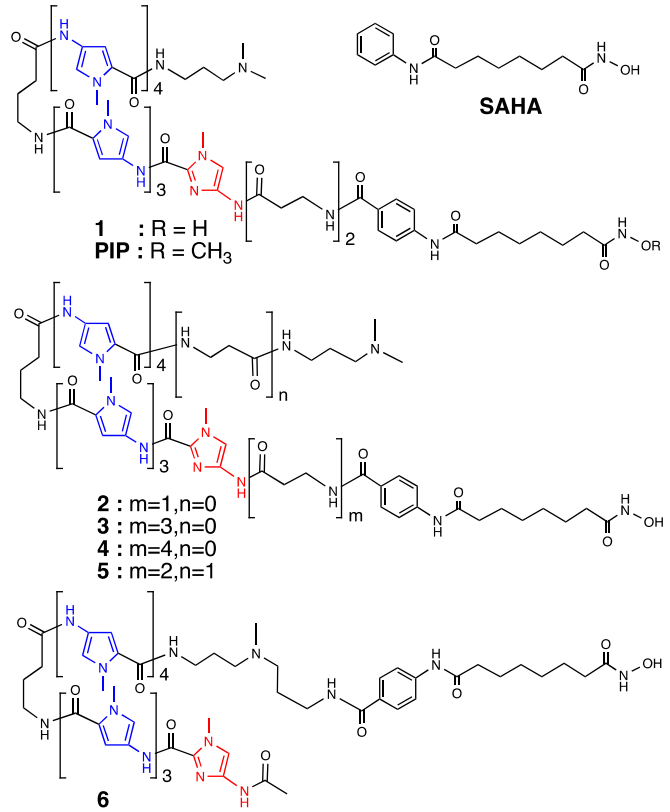

Figure 1. Chemical structures of SAHA and the synthetic PI polyamide–SAHA conjugate **1**-**6**.

2. Synthesis of derivatives of PI polyamide–SAHA conjugate 1

In this study, we designed five derivatives of PI polyamide–SAHA conjugate **1** to target a specific six-base-pair sequence according to the binding rule for PI polyamides (Fig. 1). We chose to substitute five SAHA moieties at the *N*-tail with a double β-alanine linker and one SAHA moiety at the *C*-tail with an *N*1-(3-aminopropyl)-*N*1-methylpropane-1,3-diamine linker in the hairpin PI polyamides. To investigate the effects of hybridization on the PI polyamide conjugates, all the PI polyamides were designed with different linker distances between the PI polyamides and SAHA.

Four PI polyamide–SAHA conjugates (**2**, **3**, **4**, and **5**) were synthesized by Fmoc solid-phase synthesis using an oxime resin and subsequent 3-(dimethylamino)-1-propylamine treatment, followed by aminolysis under a 50% (v/v) NH_2OH aqueous solution. SAHA conjugate **6** was also synthesized by Fmoc solid-phase synthesis using an oxime resin. After *N*1-(3-aminopropyl)-*N*1-methylpropane-1,3-diamine treatment, the *C*-terminal PI polyamide was coupled with (8-methoxy-8-oxooctanamido)benzoic acid to produce **6** by aminolysis. Purity and characterization of compounds were done as mentioned before[24]. PI polyamide with non-functional SAHA moeity (PIP) is used as the control.

After HPLC purification, each PI polyamide–SAHA conjugate was confirmed by ESI–TOF–MS, and stored before the analysis of its biological activity. The cytotoxicity of conjugates **1**–**6** was assayed as described previously[24], and the cells were almost fully viable at the working concentration of the conjugates (100 nM) (Fig. 2). The *in vitro* HDAC inhibitory activity of these conjugates also did not differ significantly.

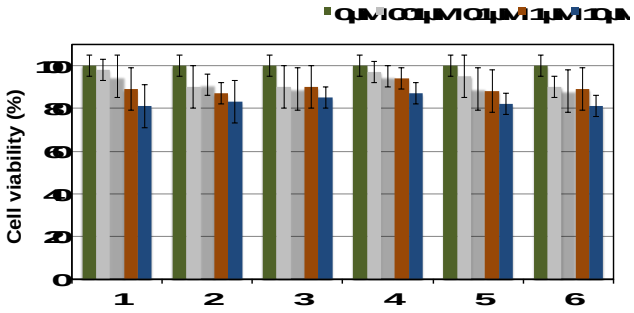

Figure 2. Cytotoxicity assay of PI polyamide SAHA conjugate **1**-**6**. Cell viability of MEF was measured after 24 h treatment of the above effectors with various concentrations of 0 μM, 0.01 μM, 0.1 μM, 1 μM and 10 μM indicated by bars in green, gray, yellow, red and blue, respectively. Each bar represents mean ± SD from 12 wells.

3. Evaluation of the PI polyamide–SAHA conjugates

3.1. *Effect of PI polyamide–SAHA conjugates on* Oct-3/4

Six PI polyamide–SAHA conjugates (**1**–**6**) were screened for their effects on the expression of factors responsible for the induction of iPSCs. Each PI polyamide–SAHA conjugate was adjusted to a final concentration of 100 nM in 0.1% DMSO and applied individually to MEF cells at 37 °C for 24 h, followed by RNA isolation and cDNA synthesis, as described in the experimental section. After normalization to house keeping genes, the relative expression level of each gene was analyzed, taking the gene expression in the DMSO-treated cells as 100%. Although the expression of *Oct-3/4* was increased in MEF cells treated with all the PI polyamide–SAHA conjugates, differential effects on the expression of this gene were observed. A marked increase in the endogenous expression of *Oct-3/4* was observed when the PI polyamide was attached to SAHA by a linker of two or more β-alanines, and the maximum increase of about 4.5-fold was observed with a three-β-alanine linker (Fig. 3, bar 3). However, when the number of β-alanines in the linker was increased to four, an increase of about 3.6-fold in the expression of *Oct-3/4* was observed, whereas the standard PI polyamide–SAHA conjugate **1** showed an increase of only about 3.1-fold (Fig. 3, bars 1 and 4, respectively). Interestingly, both the variants of the PI polyamide in the *C*-terminal region with one additional β-alanine linker (**5**) and the variant attached to SAHA instead of to the *N*-terminal region (**6**) increased *Oct-3/4* gene expression by only about two-fold (Fig. 3, bars 5 and 6, respectively). It is important to note here that the PIP and SAHA alone had almost no effect (Fig. 3, bars PIP and SAHA, respectively). Because SAHA conjugate **2** induced an increase of only about two-fold, conjugate **3** linked to SAHA by three β-alanines was considered the optimal structure for induction of *Oct-3/4* expression.

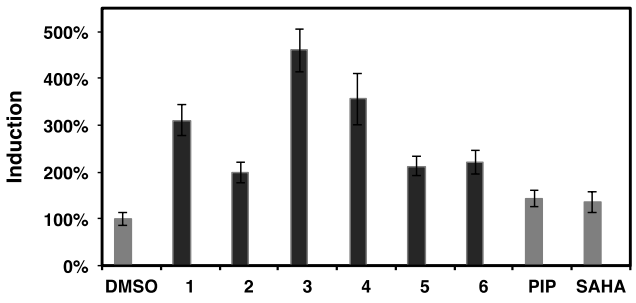

Figure 3. Distinctively programmed SAHA conjugates differentially upregulate the expression of *Oct-3/4*. qRT–PCR analysis of the expression levels of *Oct-3/4* after treatment with 100 nM of the effectors for 24 h. Light

gray bars represent the controls used: 0.1% DMSO, PI polyamide conjugates without SAHA, and SAHA alone. Dark gray bars represent the expression profiles of the endogenous genes induced with PI polyamide-SAHA conjugates **1–6**. Each bar represents the mean ± SD of a 24-well plate.

3.2. *Effects of PI polyamide SAHA conjugates on Nanog and Sox2*

Together with *Oct-3/4*, pluripotency is co-regulated through the activation of *Sox2* and *Nanog*, which are involved in the core transcriptional network[25]. Therefore, the effects of conjugates **1**–**6** on the endogenous expression of both *Nanog* and *Sox2* were studied, as mentioned above. Conjugate **1** was previously shown to increase *Nanog* expression by about 2.5-fold, and the expression profiles of *Nanog* in cells treated with conjugate **1** derivatives were similar to those of *Oct-3/4*, where maximum induction was observed with PI polyamide–SAHA conjugate **3** (Fig. 4, light gray bar 3). The other variants showed only a relative moderate increase of about two-fold in the expression of *Nanog* (Fig. 4, light gray bars 1, 2, 4, 5, and 6). Consistent with the previous results, the control samples containing only SAHA or the PIP had no effect on *Nanog* expression (Fig. 4, light gray bars DMSO, PIP, and SAHA).

Although conjugate **1** and its derivatives also markedly increased *Sox2* expression compared with that in the control samples, there were few or no differences in the effects of the derivatives on the *Sox2* expression profile, including the derivative with the three-β-alanine linker (Fig. 4, dark gray bars). Thus, the expression of *Nanog* and *Sox2* was upregulated by PI polyamide–SAHA conjugate **3**.

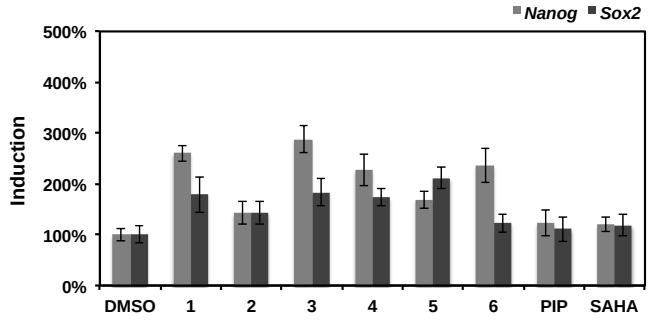

Figure 4. Effects of PI polyamide-SAHA conjugates on the endogenous expression of *Nanog* and *Sox2*. qRT–PCR analysis was performed to determine the expression profiles of *Nanog* (light gray bar) and *Sox2* (dark gray bar) after treatment with SAHA conjugates **1–6** or the controls (DMSO, SAHA only, and PI polyamide conjugates only), as described in the text. Each bar represents the mean ± SD of a 24-well plate.

3.3. Effect of PI polyamide SAHA conjugates on Klf4 and c-Myc

A precise balance of *Klf4* and *c-Myc* transcriptional network and core pluripotency genes is essential for the successful reprogramming of the somatic genome[26]. Conjugate **1** showed little or no effect on the expression of either *Klf4* or *c-Myc*[24]. Interestingly, the derivatives of conjugate **1** showed different induction patterns from those observed for *Oct-3/4*, *Sox2*, and *Nanog*. Variations in the β-alanine linker did not cause any significant differences in the expression profile of *Klf4*. Surprisingly, a maximum induction of about 2.2-fold in the expression profile of *Klf4* was observed with the PI polyamide–SAHA conjugate with SAHA attached in the *C*-terminal region (**6**) (Fig. 5, light gray bar 6). A similar induction pattern, with an increase in the expression of *Klf4* of about 1.5- to 1.8-fold, was observed with the other variant PI polyamide conjugates. Another *C*-terminal variant with a β-alanine linker (**5**) showed relatively lower induction of *Klf4* (Fig. 5, light gray bar 5). However, the difference in activity is not remarkable to derive conclusion about the actual mechanism of action of these compounds

The PI polyamide attached to SAHA by one β-alanine (**2**) had almost no effect on *c-Myc* expression (Fig. 5, dark gray bar 2), whereas a slight increase of about 1.5-fold in its expression profile was observed with the other derivatives (Fig. 5, dark gray bars). A maximum increase of about 1.8-fold was induced by the *C*-terminal variant with a β-alanine linker, but this increase did not differ significantly from those induced by the other variants. Nevertheless, this increase was considerable compared with the control values (Fig. 5, dark gray bar 5).

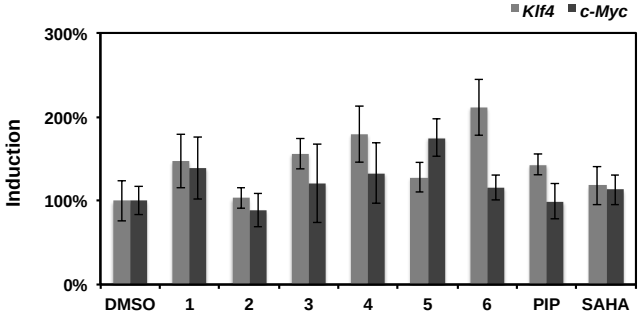

Figure 5. Effects of PI polyamide-SAHA conjugates on the expression of *Klf4* and *c-Myc*. qRT-PCR analysis was performed with SAHA conjugates **1**–**6** and controls (DMSO, SAHA only, and PI polyamide only), as described in the text, to obtain the expression profile of *Klf4* (light gray bar) and *c-Myc* (dark gray bar). Each bar represents the mean ± SD of a 24-well plate.

4. Discussion

The directed chemical reprogramming of the somatic genome to generate iPSCs offers "paradigm-shifting opportunities", including personalized renewable sources of cells for practical cell therapies, regenerative medicine, disease modeling, and prognostic toxicology applications. Therefore, transcriptional activators that can force the endogenous expression of the genes associated with pluripotency have received immense attention. Recently, small molecules that can replace reprogramming factors have been vigorously studied [27, 28]. A recent report on the generation of iPSCs with a single gene, *Oct-4*, and small molecules suggests that generation of iPSCs with only small molecules is near the horizon[29]. Chromatin modifiers have already had a significant impact in improving reprogramming efficiency. Valproic acid and sodium butyrate notably increased either the three-factor reprogramming efficiency in both mouse and human cells or the two-factor reprogramming efficiency in human fibroblasts. Interestingly, SAHA, a potent HDAC inhibitor with a broad spectrum of epigenetic activities, displayed relatively mild effects[21,22]. However, at least a single transcription factor, *Oct-3/4*, is still required, and together with *Nanog*, it plays an integral role in establishing and maintaining pluripotency. Considering the progress in iPSC technology thus far, the precise activation of the core pluripotency genes should be a viable way of improving the efficiency of reprogramming somatic cells to generate iPSCs[30].

In this context, the use of small selective DNA-binding molecules that can induce the expression of core pluripotency genes could be a reasonable strategy to overcome the rate-limiting step of somatic cell reprogramming. We have shown that a new type of PI polyamide conjugated to the HDAC inhibitor SAHA can selectively acetylate the promoter region of the p16 tumor suppressor gene in HeLa cells[23]. Because histone modification is associated with pluripotency, iPSC factors were

chosen as the candidate genes for screening studies of the new sequence-specific PI polyamide-SAHA conjugates (**A**–**P**). Unprecedentedly, we identified certain conjugates that could significantly upregulate the endogenous expression of iPSC factors in a differential manner[24]. Among them, PI polyamide-SAHA conjugate **1**, previously designated **E**, notably induced the expression of the core pluripotency genes *Oct-3/4* and *Nanog*. A chromatin immunoprecipitation analysis (ChIP) of the *Oct-3/4* promoter region clearly suggested that conjugate **1** induced the enrichment of activation markers (H4Kac, H3K9ac, H3K14ac, and H3K4me3) and moderately reduced the expression of markers of repression (H3K9me3 and H3K27me3), whereas the control SAHA conjugate, SAHA, and DMSO had no such effect[24]. The sequence specificity of conjugate **1** was further confirmed with a ChIP analysis of the *Nanog* region, which clearly indicated that this transcriptional activation occurs only in the promoter and transcribed region of the gene.

Unlike other small molecules that have been used in the generation of iPSCs, PI polyamide–SAHA conjugates are programmable because of the presence of tunable sites, which facilitate the covalent attachment of functional molecules[31,32]. In this study, we chose conjugate **1** and synthesized a series of derivatives that included modifications to the number of β-alanine molecules in the linker used to conjugate the PI polyamide with SAHA (conjugates **1**–**4**) and variants in the *C*-terminal region (conjugates **5** and **6**). Three-β-alanine linker caused maximum expression of *Oct-3/4* (Fig. 3, bars 1–4). While similar patterns were also observed for *Nanog* and *Sox2*, the differences between these two genes in the degree of induction were not striking (Fig. 4, bars 1–4), which suggests that *Oct-3/4* is the direct target of conjugate **1**. In contrast, *C*-terminal variants showed relatively lower induction values (Figs. 3 and 4, bars 5 and 6). Interestingly, a reversal in the pattern of induction was observed for *Klf4*, and the *C*-terminal variant conjugate 6 produced relatively better induction than that with the various β-alanine variants (Fig. 5). Although it is known that *Klf4* and *c-Myc* act in a different pathway from the core pluripotency gene network[26], the induction values alone is insufficient to suggest a different operating mechanism. Also, the actual binding site of our PI polyamide-SAHA conjugate is yet to be clarified. Nevertheless, the induction ability of our PI polyamide-SAHA conjugates in just 24 h could be employed to overcome the limitation of duration in reprogramming process.

5. Conclusion

Our results clearly suggest that a PI polyamide linked to SAHA by three β-alanine groups increases the expression of *Oct-3/4* and *Nanog*. Because only PI polyamide–SAHA conjugate **1** and not the other PI polyamide–SAHA conjugates increased the expression of *Oct-3/4* and *Nanog*, sequence specificity is inferred to be the mechanism underlying this upregulation. Differential patterns of induction were also clearly observed with PI polyamide–SAHA conjugates **2**–**6**, which suggests that a second generation of PI polyamide–SAHA conjugate **1** derivatives could be generated to enhance the reprogramming efficiency of somatic cells to iPSCs. Programmable DNA-binding molecules have been explored as possible candidates for artificial genetic switches. However, their low efficiency, attributable to their lack of specificity and an affinity for methylated DNA sequences, has been a major concern[13]. Using a predictive genomewide binding study across the entire sequence space and different classes of proteins and engineered DNA-binding molecules, Carlson et al. showed that the specificities of PI polyamides surpass those of natural DNA-binding proteins[33]. We have also demonstrated that PI polyamides have about a three-fold higher binding affinity for methylated CpG islands. Our recent report on the epigenetic activation of pluripotency genes by our library of designed SAHA conjugates and the scope of improvements shown in this study further substantiate the hypothesis that PI polyamide–SAHA conjugates can be programmed for development as artificial genetic “ON” switches.

6. Experiments

6.1. General

Reagents and solvents were purchased from standard suppliers and used without further purification. ^{1}H-NMR spectra were recorded with JEOL JNM ECA-600 spectrometer operating at 600 MHz for ^{1}H NMR and tetramethylsilane was used as an internal standard. Proton NMR spectra were recorded in parts per million (ppm) downfield relative to tetramethylsilane. The following abbreviations apply to spin multiplicity: s (singlet), d (doublet), t (triplet), m (multiplet), br (broaded). High-performance liquid chromatography (HPLC) purification was performed with a JASCO CCPS HPLC pump, a JASCO UV8020 HPLC UV/VIS detector and a Chemcobond 5-ODS-H reversed phase column (10 x150 mm) in 0.1% TFA in water with CH_3CN as eluent at a flow rate of 3.0 mL/min, and a linear gradient elution of 40–60% CH_3CN over 40 min with detection at 254 nm. Electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) were produced on a BioTOF II (Bruker Daltonics) mass spectrometry using a positive ionization mode. All DNA fragments, 5’-labeled primers and cold primers were purchased from Sigma–Aldrich. Thermo sequence core sequencing kit was purchased from GE Healthcare. Polymerase chain reaction (PCR) was performed on an iCyclear (BIO-RAD). Machine-assisted PI polyamide syntheses were performed on a peptide synthesizer, PSSM-8 (SHIMADZU) in a stepwise reaction by Fmoc solid phase protocol.

6.2. Synthesis of PI polyamide SAHA conjugates

All machine-assisted polyamide syntheses were performed on a PSSM-8 peptide synthesizer (Shimadzu, Kyoto) with a computer-assisted operation system at 40 mg of oxime resin (0.5 mmol/g, 200~400 mesh) by using Fmoc solid-phase chemistry according to reported procedure[24].

After the conversion to SAHA conjugates, HPLC purification (0.1%TFA-CH_3CN 40-60% linear gradient, 0-40 min, 254 nm) was used to obtain the desired **1**-**6**.

1[24]; ESI-TOF-MS(positive) m/z calcd for $C_{77}H_{96}N_{24}O_{15}^{2+}$ ［M＋2H］$^{2+}$ 799.36; found 799.16.

2; ESI-TOF-MS(positive) m/z calcd for $C_{74}H_{91}N_{23}O_{14}^{2+}$ ［M＋2H］$^{2+}$ 763.86; found 763.60.

3; ^{1}H NMR (600 MHz, [D_6]DMSO): δ= 10.33 (*s*, 1H), 10.28 (*s*, 1H), 10.07 (*s*, 1H), 10.00 (*s*, 1H), 9.94 (*s*, 1H), 9.93 (*s*, 1H), 9.90 (*s*, 2H), 9.89 (*s*, 1H), 9.84 (*s*, 1H), 9.23 (*brs*, 1H), 8.36 (*brt*, 1H), 8.15 (*brt*, 1H), 8.05 (*brt*, 1H), 7.99 (*brt*, 1H), 7.92 (*brt*, 1H), 7.76 (*d*, *J* =8.9Hz, 2H), 7.63 (*d*, *J* =8.9Hz, 2H), 7.39 (*s*, 1H), 7.27 (*s*, 1H), 7.22 (*s*, 2H), 7.17 (*s*, 3H), 7.15 (*s*, 1H), 7.09 (*s*, 1H), 7.07 (*s*, 2H), 7.00 (*s*, 1H), 6.95 (*s*, 1H), 6.91 (*s*, 1H), 6.88 (*s*, 1H), 3.95 (*s*, 3H), 3.86 (*s*, 3H), 3.85 (*s*, 6H), 3.84 (*s*, 3H), 3.83 (*s*, 3H), 3.81 (*s*, 3H), 3.80 (*s*, 3H), 3.22-3.31 (*m*, 12H), 3.06 (*m*, 4H), 2.81 (*s*, 3H), 2.79 (*s*, 3H), 2.27-2.33 (*m*, 6H), 2.22 (*t*, *J* =7.6Hz, 2H), 1.93 (*t*, *J* =7.6Hz, 2H), 1.84 (*m*, 2H), 1.79 (*m*, 2H), 1.56 (*m*, 2H), 1.48 (*m*, 2H), 1.26 (*m*, 2H); ESI-TOF-MS(positive) m/z calcd for $C_{80}H_{101}N_{25}O_{16}^{2+}$ ［M＋2H］$^{2+}$ 834.91; found 834.67.

4; ESI-TOF-MS(positive) m/z calcd for $C_{83}H_{106}N_{26}O_{17}^{2+}$ ［M＋2H］$^{2+}$ 870.41; found 870.19.
5; ESI-TOF-MS(positive) m/z calcd for $C_{80}H_{101}N_{25}O_{16}^{2+}$ ［M＋2H］$^{2+}$ 834.91; found 834.70.
6; ESI-TOF-MS(positive) m/z calcd for $C_{75}H_{93}N_{23}O_{14}^{2+}$ ［M＋2H］$^{2+}$ 770.86; found 770.63.

Supplementary Material

Experimental details about cytotoxicity, cell culture, HPLC chromatogram of compounds and quantification of gene expression is submitted as supplementary material along with this manuscript.

Acknowledgments

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References and notes

1. Winslow, R. L.; Boguski, M. S. Circ. Res. 2003, 92, 953.
2. Watson, J. D. Science 1990, 248, 44.
3. Chin, L.; Anderson, J. N.; Futreal, P. A. Nat. Med. 2011, 17, 297.
4. Wu, Z. Q.; Belanger, G.; Brennan, B. B.; Lum, J. K.; Minter, A. R.; Rowe, S. P.; Plachetka, A.; Majmudar, C. Y.; Mapp, A. K. J. Am. Chem. Soc. 2003, 125, 12390.
5. Ptashne, M.; Gann, A. Nature 1997, 386, 569.
6. Juven-Gershon, T.; Kadonaga, J. T. Dev. Biol. 2010, 339, 225.
7. Blancafort, P.; Magnenat, L.; Barbas, C. F.3rd. Nat. Biotechnol. 2003, 21, 269.
8. Gonzalez, B.; Schwimmer, L. J.; Fuller, R. P.; Ye, Y.; Asawapornmongkol, L.; Barbas, C. F. Nat. Protoc. 5 2010, 5, 791.
9. Grizot, S.; Smith, J.; Daboussi, F.; Prieto, J.; Redondo, P.; Merino, N.; Villate, M.; Thomas, S.; Lemaire, L.; Montoya, G.; Blanco, F. J.; Paques, F.; Duchateau, P. Nucleic Acids Res. 2009, 37, 5405.
10. Zhang, F.; Cong, L.; Lodato, S.; Kosuri, S.; Church, G.; Arlotta, P. Nat. Biotechnol. 2011, 29, 149.
11. Baltimore, D. Our genome unveiled, Nature 2001, 409, 814.
12. Jenuwein, T.; Allis, C. D. Science 2001, 293, 1074.
13. Bogdanove, A. J.; Voytas, D. F. Science 2011, 333, 1843.
14. Takahashi, K.; Yamanaka, S. Cell 2007, 126, 663.
15. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Cell 2007, 131, 861.
16. Park, I. H.; Arora, N.; Huo, H.; Maherali, N.; Ahfeldt, T.; Shimamura, A.; Lensch, M. W.; Cowan, C.; Hochedlinger, K.; Daley, G. Q. Cell 2008, 134, 877.
17. Tsuji, O.; Miura, K.; Okada, Y.; Fujiyoshi, K.; Mukaino, M.; Nagoshi, N.; Kitamura, K.; Kumagai, G.; Nishino, M.; Tomisato, S. Proc Natl Acad Sci U S A. 2010, 107, 12704.
18. Kim, K.; Doi, A.; Wen, B.; Ng, K.; Zhao, R.; Cahan, P.; Kim, J.; Aryee, M. J.; Ji, H.; Ehrlich, L. I. Nature 2010, 467, 285.
19. Maekawa, M.; Yamaguchi, K.; Nakamura, T.; Shibukawa, R.; Kodanaka, I.; Ichisaka, T.; Kawamura, Y.; Mochizuki, H.; Goshima, N.; Yamanaka, S. Nature 2011, 474, 225.
20. Huangfu, D.; Maehr, R.; Guo, W.; Eijkelenboom, A.; Snitow, M.; Chen, A. E.; Melton, D. A. Nat Biotechnol. 2008, 26, 795.
21. Mali, P.; Chou, B. K.; Yen, J.; Ye, Z.; Zou, J.; Dowey, S.; Brodsky, R. A.; Ohm, J. E.; Yu, W.; Baylin, S. B.; Yusa, K. S.; Bradley, A.; Meyers, D. J.; Mukherjee, C.; Cole, P. A.; Cheng, L. Stem Cells 2010, 28, 713.
22. Selvaraj, V.; Plane, J. M.; Williams, A. J.; Deng, W. Trends in Biotech. 2010, 28, 214.
23. Ohtsuki, A.; Kimura, M. T.; Minoshima, M.; Suzuki, T.; Ikeda, M.; Bando, T.; Nagase, H.; Shinohara, K.; Sugiyama, H. Tetrahedron Lett. 2009, 50, 7288.
24. Pandian, G. N.; Shinohara, K.; Ohtsuki, A.; Nakano, Y.; Yamada, Y.; Watanabe, A.; Terada, N.; Sato, S.; Morinaga, H.; Sugiyama, H. ChemBioChem. 2011, 12, 2822.
25. Loh, Y. H.; Wu, Q.; Chew, J. L.; Vega, V. B.; Zhang, W.; Chen, X.; Bourque, G.; George, J.; Leong, B.; Liu, L. Nat Genet. 2006, 38, 431.
26. Rodolfa, K. T.; Eggan, K. Cell 2006, 126, 652.
27. Shi, Y.; Desponts, C.; Do, J. T.; Hahm, H. S.; Scholer, H. R.; Ding, S. Cell Stem Cell 3 (2008) 568–574.
28. Li, Y.; Zhang, Q.; Yin, X. Cell Research 2010, 21, 196.
29. Ichida, J. K.; Blanchard, J.; Lam, K.; Son, E. Y.; Chung, J. E.; Egli, D.; Loh, K. M.; Carter, A. C.; Di Giorgio, F. P.; Koszka, K.; Huangfu, D.; Akutsu, H.; Liu, D. R.; Rubin, L. L.; Eggan, K. A. Cell Stem Cell 2009, 5, 491.
30. Yamanaka, S. Cell. 2009, 137, 13.
31. Jacobs, C. S.; Dervan, P. B. J Med Chem. 2009, 52, 7380.
32. Wang, C. C.; Ellervik, U.; Dervan, P. B. Bioorg Med Chem. 2001, 9, 653.
33. Carlson, C. D.; Warren, C. L.; Hauschild, K. E.; Ozers, M. S.; Qadir, N.; Bhimsaria, D.; Lee, Y.; Cerrina, F.; Ansari, A. Z. Proc. Natl. Acad. Sci. USA. 2010, 107, 4544.

Graphical Abstract

Development of programmable small DNA binding molecules with epigenetic activity for induction of core pluripotent genes

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