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Soluble expression and stability enhancement of transcription factors using 30Kc19 cell-penetrating protein

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Abstract Transcription factors have been studied as an important drug candidate. Ever since the successful generation of induced pluripotent stem cells (iPSCs), there has been tremendous interest in reprogramming transcription factors. Because of the safety risks involved in a virus-based approach, many researchers have been trying to deliver transcription factors using nonintegrating materials. Thus, delivery of transcription factors produced as recombinant proteins in *E. coli* was proposed as an alternative method. However, the low level of soluble expression and instability of such recombinant proteins are potential barriers. We engineered a *Bombyx mori* 30Kc19 protein as a fusion partner for transcription factors to overcome those problems. We have previously reported that 30Kc19 protein can be produced as a soluble form in *E. coli* and has a cell-penetrating property and a protein-stabilizing effect. Transcription factors fused with 30Kc19 (Oct4-30Kc19, Sox2-30Kc19, c-Myc-30Kc19, L-Myc-30Kc19, and Klf4-30Kc19) were produced as recombinant proteins. Interestingly, Oct4 and L-Myc were expressed as a soluble form by conjugating with 30Kc19 protein, whereas Oct4 alone and L-Myc alone aggregated. The 30Kc19 protein also enhanced the stability of transcription factors both in vitro and in cells. In addition, 30Kc19-conjugated transcription factors showed rapid delivery into cells and transcriptional activity significantly increased. Overall, 30Kc19 protein conjugation simultaneously enhanced soluble expression, stability, and transcriptional activity of transcription factors. We propose that the conjugation with 30Kc19 protein is a novel approach to solve the technical bottleneck of gene regulation using transcription factors.

Keywords 30Kc19 protein · Cell-penetrating property · Recombinant protein · Transcription factor · Soluble expression · Protein stability

Introduction

A transcription factor is known as a regulator that localizes into the nucleus and controls gene expression by binding to specific DNA sequences (Boulikas 1994). Since the generation of induced pluripotent stem cells (iPSCs) using four defined transcription factors (Oct4, Sox2, c-Myc, and Klf4), many have taken an interest because transcription factors have clinical significance as a therapeutic gene modulator (Takahashi et al. 2007; Takahashi and Yamanaka 2006). Defined transcription factors, which are delivered by viral vectors, can induce somatic cells into becoming pluripotent stem cells. However, a virus-based approach may cause tumorigenicity due to integration into the host cell genome (Maherali and Hochedlinger 2008). As a solution, there have been many trials to evade viral transduction by using

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nonintegrating plasmids (Chou et al. 2011). PiggyBac transposons (Woltjen et al. 2009). and small-molecule compounds (Hou et al. 2013). Although plasmids and transposons can reduce the risks, they still have potency for genomic integration. Besides, molecule compounds were able to reprogram mouse cells but were insufficient in reprograming human cells. Thus, a protein-based approach is a practical method for clinical use that can limit safety risks and has many advantages (Cho et al. 2010; Kim et al. 2009). For example, neuronal precursor cells (NPCs) derived from protein-based iPSCs showed high expansion, whereas NPCs from virus-based iPSCs exhibited early senescence and apoptosis (Rhee et al. 2011).

There were many trials to express transcription factors as recombinant proteins for the exploration of their mechanisms and the application as clinical drugs (Mossakowska 1998). *Escherichia coli* (*E. coli*) has been used commonly for the production of recombinant proteins due to its high productivity, low production cost, and ease of product isolation (Sorensen and Mortensen 2005). However, there are some technical bottlenecks in the production of transcription factor from *E. coli*. For instance, the recombinant proteins are expressed as aggregate forms in many cases. As a result, it requires additional solubilization and refolding steps which increases production cost and decreases yield (Chan et al. 2013; Dyson et al. 2004). In addition, there is the risk that the protein may lose the original bioactivity after the refolding process (Chang et al. 2014; Smialowski et al. 2007). Methods to increase solubility have been reported by optimizing *E. coli* culture conditions (Yang et al. 2009) or through the use of a buffer system (Golovanov et al. 2004).

In addition to the production of transcription factors, cell-penetrating peptides (CPPs) are required for the intracellular delivery of recombinant transcription factors, and many researchers have used various CPPs, such as TAT (Frankel and Pabo 1988). Antp (Perez et al. 1992). and poly-arginine (El-Sayed et al. 2009). TAT was reported to have a higher bioactivity than others (Zhang et al. 2012). however, there is a limitation for clinical applications, as TAT is an HIV virus-derived cell-penetrating peptide. Many CPPs have been used only for delivery, and they did not affect transcription factors for soluble expression or stability. Therefore, most transcription factors with CPPs were expressed as an inclusion body from *E. coli* making the purification process difficult or causing the loss of bioactivity (Yang et al. 2009).

In our previous study, we introduced silkworm hemolymph (SH) as a substitute for fetal bovine serum (Ha et al. 1996) and reported that SH increased host cell longevity (Ha and Park 1997). Purified 30K proteins from SH had an interesting role in inhibiting apoptosis in various cells (Choi et al. 2002; Kim et al. 2003; Kim and Park 2003; Kim et al. 2001; Kim et al. 2004; Park et al. 2003; Rhee et al. 1999; Rhee et al. 2002; Rhee and Park 2000). The most abundant component among 30K proteins, 30Kc19 protein, delivered protein cargos into cells (Park et al. 2012a, 2014b) by a dimerization mechanism (Park et al. 2014a) and enhanced enzyme stability (Park et al. 2015; Park et al. 2012b). Furthermore, 30Kc19 protein and human serum albumin nanoparticles were generated and successfully delivered enzyme into cells or organs with low cytotoxicity (Lee et al. 2014). Here, we have used 30Kc19 cell-penetrating protein as a novel fusion partner of transcription factors which can be used in protein-based iPSC generation. We anticipate that a 30Kc19 protein could deliver a transcription factor into cells and also solve problems that normally arise in the production of transcription factors, such as low soluble expression and protein instability (Singh and Panda 2005). Thus, we propose that the multifunctional properties of 30Kc19 protein can be applied to general transcription factors and overcome several hurdles when used as recombinant proteins.

Materials and methods

Plasmid construction and protein expression

The *30Kc19* gene was constructed using *Bombyx mori* total RNA (Kim et al. 2001) and amplified by polymerase chain reaction (PCR). PCR product was inserted into a pET-23a vector (Novagen, Madison, WI, USA) at *Eco*R1 and *Xho*1 site. Five transcription factors including Oct4 (Addgene plasmid # 17217), Sox2 (Addgene plasmid # 17218), c-Myc (Addgene plasmid # 17220), Klf4 (Addgene plasmid # 17219), and L-Myc (Addgene plasmid # 26022) were also inserted into the vector at *Bam*H1, *Eco*R1. Each vector was designed with a T7 tag at the N-terminus for immunoassay and His tag at the C-terminus for purification. Each vector was transformed to *E. coli* BL21 (Novagen), and cells were cultured in LB medium with 100 μg/ml ampicillin. When OD_{600} reached 0.6 at 37 °C, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM. For protein expression, the cells were further incubated at 27 or 37 °C.

Protein purification by fast protein liquid chromatography

Harvested cells were disrupted by ultra-sonication in a lysis buffer (20 mM Tris-HCl, 0.5 M NaCl, 20 mM imidazole, pH 8.0) and separated by centrifugation (12,000 rpm). The soluble proteins in the supernatant were collected and loaded on a HisTrap HP column (GE Healthcare, Uppsala, Sweden) using FPLC (GE Healthcare). The washing buffer (20 mM Tris-HCl, 0.5 M NaCl, 50 mM imidazole, pH 8.0) was placed into the column to remove unbound proteins. Finally, target proteins were eluted with the elution buffer (20 mM Tris-HCl, 0.5 M NaCl, 350 mM imidazole, pH 8.0) and dialyzed against

Dulbecco's modified eagle medium (DMEM, Gibco, Invitrogen, Carlsbad, CA, USA).

Western blot analysis

Soluble transcription factors were analyzed using soluble fraction in *E. coli* lysate. To check the stability of soluble proteins, seven proteins (Oct4-30Kc19, Sox2-30Kc19, Klf4-30Kc19, Sox2, Klf4, Sox2-R9, and Klf4-R9) were purified and incubated at 37 °C. The samples were separated thru 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membrane (GE Healthcare). The membranes were blocked with 5 % skim milk in TBS with 0.1 % tween 20. Anti-T7 primary antibody (Abcam, Cambridge, UK) and anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody (Millipore, Bedford, MA, USA) were used for immunoblot analysis. Luminata Forte Western HRP Substrate (Millipore) was used as substrate of HRP. The band was visualized by G:BOX Chemi XL system (Syngene, Cambridge, UK), and the band intensities were quantified using ImageJ software.

Live cell imaging and immunofluorescence microscopy

For live cell imaging assay, recombinant proteins were labeled with an Alexa Fluor® 488 protein labeling kit (Invitrogen) according to the manufacturer's instructions. Human dermal fibroblast (HDF, ATCC # PCS-201-010) cells were treated with 20 μg/ml of labeled proteins. For immunofluorescence assay, anti-T7 primary antibody (1:200) and anti-rabbit Alexa 488 or 594-conjugated secondary antibody (1:400) were used on cells after treatment with 10 μg/ml of protein for 24 or 48 h. Hoechst 33342 (Life Technologies, Gaithersburg, MD) was stained on nucleus and observed using confocal microscopy (Olympus, Lake Success, NY, USA).

Luciferase assay

pGL3-Klf4 reporter plasmid and pRL-SV40 (Addgene plasmid # 27163) were delivered into HEK293 cells using Lipofectamine® 3000 reagent (Invitrogen) according to the manufacturer's instructions. One day after the transfection, cells were treated with proteins. A Dual-Glo® luciferase assay system (Promega, Madison, WI, USA) was used for measurement of luciferase. On a 96-well plate (Nunc Lab-Tek, Thermo Scientific, Rockford, IL, USA), the cells were treated with proteins for 4 h and then washed with DMEM. Twenty microliters of a lysis reagent was added in 20 μl of DMEM medium. After 10 min of incubation, the firefly luminescence was measured using Luminometer (Thermo Scientific). Then 20 μl of stop reagent was added before measurement. The ratio of Firefly/*Renilla* luminescence was calculated, and each well was normalized from a control well.

Cell viability assay

To assess the toxicity of 30Kc19-conjugated protein, HDF cells were treated with proteins (Oct4-30Kc19, Sox2-30Kc19, c-Myc-30Kc19, and Klf4-30Kc19) in a 96-well plate. The same concentrations of each protein, 2, 5, 10, and 15 μg/ml (total protein 8, 20, 40, and 60 μg/ml) were added for 12 or 24 h. After treatment, cells were washed three times with PBS and treated with MTT solution for 4 h then solubilized for 12 h at 37 °C. Absorbance at 420 nm was measured for the determination of cell viability.

Results

Soluble expression and purification of transcription factors

Five transcription factors (Oct4, Sox2, c-Myc, Klf4, and L-Myc) were selected as they are most popular regulators and have been extensively studied since after the discovery of iPSCs (Takahashi and Yamanaka 2006). We assumed that a 30Kc19 cell-penetrating protein would be a good strategic fusion partner for unstable transcription factors. That is, a 30Kc19 protein can simultaneously enhance soluble expression, increase stability, and enhance delivery of transcription factors into cells (Fig. 1).

First of all, the effect of 30Kc19 protein conjugation to transcription factors on soluble expression was determined (Fig. 2). Transcription factors were cloned in the pET-23a vector for recombinant protein expression in *E. coli*. Each factor was comprised with a T7 tag at the N-terminus for immunoassay and a His tag at the C-terminus for purification using affinity chromatography (Fig. 2a). For the delivery of protein into cells, each factor was fused with nine-arginine (R9) or 30Kc19. Transcription factor alone or with 30Kc19 was expressed in *E. coli*, and the lysates were separated into soluble and insoluble fractions. Total lysate and soluble fraction were then analyzed by SDS-PAGE and Western blot. In previous studies, most transcription factors were demonstrated to have poor solubility and require a solubilization process (Hu et al. 2012; Pan et al. 2010; Zhang et al. 2012; Zhou et al. 2009). When Oct4 alone was expressed, it was expressed mainly as an inclusion body, and the level of soluble form was extremely low (Fig. 2b). Interestingly, a considerable amount of Oct4 conjugated with 30Kc19 protein was expressed in soluble fraction, meaning that 30Kc19 protein enhanced soluble expression. For Sox2 and Klf4, we observed that some of those transcription factors were expressed as soluble proteins

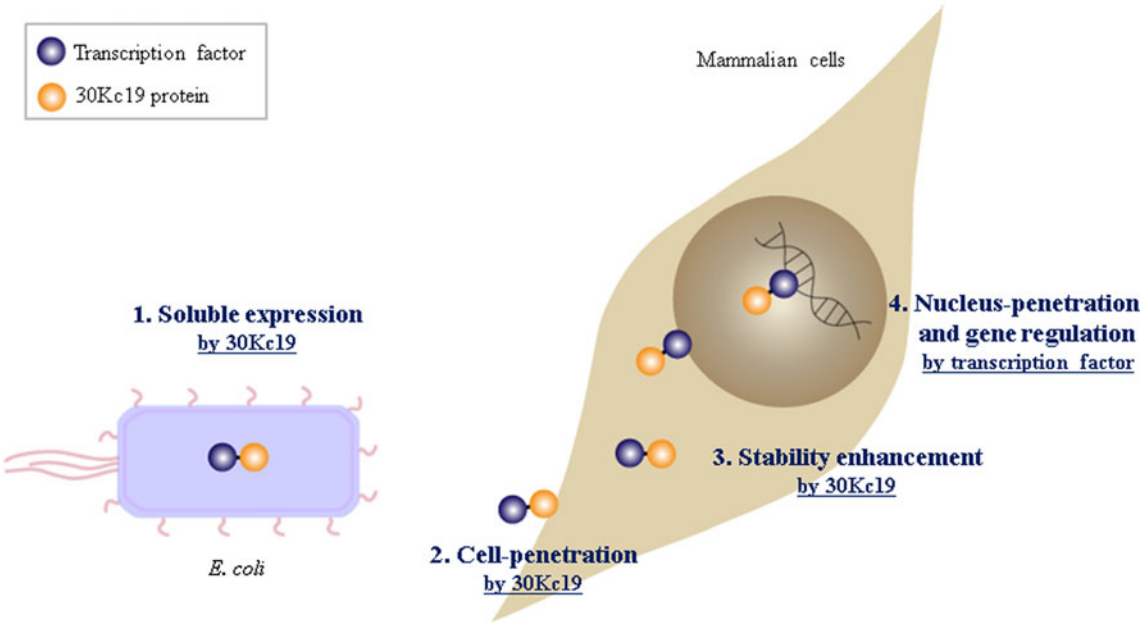

Fig. 1 A schematic illustration of gene regulation by transcription factor-conjugated 30Kc19 protein

in our expression system as shown in Fig. 2b. The relative protein expression level of each protein is shown in Fig. 2c.

To assess the effect of CPPs (R9 and 30Kc19) on soluble expression, transcription factors conjugated with R9 or 30Kc19 were expressed at 27 or 37 °C and the soluble fraction of each protein was analyzed by Western blot. As shown in Fig. 2d, 30Kc19 promoted the production of Oct4 more as a soluble protein when expressed at either 27 or 37 °C, while R9 did not influence Oct4 solubilization. In accordance with Fig. 2d, Sox2 and Klf4 were expressed as soluble proteins regardless of CPP type. While c-Myc was expressed as insoluble protein in any situation (Fig. S1), another Myc family member, L-Myc (Nakagawa et al. 2008; Nakagawa et al. 2010). was highly expressed as a soluble protein when it was fused with 30Kc19 protein, whereas L-Myc alone showed a lower expression level and was not produced in soluble form (Fig. S2a). With R9, a small amount of L-Myc was expressed as soluble protein at 27 °C (Fig. S2b). Overall, these results demonstrate that 30Kc19 protein enhanced the soluble expression of Oct4, Sox2, and L-Myc, which became 7.0, 2.9, and 23.8 times its original amount, respectively.

To further explore the role of the 30Kc19 protein conjugation on recombinant transcription factors, soluble transcription factors were purified using affinity chromatography. The size and amount of purified soluble proteins were confirmed by Coommasie blue staining (Fig. 3a) or Western blot (Fig. 3b). As shown in Fig. 3, each of the purified proteins was confirmed for their proper size. The results of purified Oct4 alone are not shown as there was insufficient amount of original soluble Oct4 as mentioned in Fig. 2. The amounts of purified soluble Sox2 and Klf4, without any conjugations, were also low. In contrast, a relatively high level of purified soluble Oct4-30Kc19, Sox2-30Kc19, and Klf4-30Kc19 was observed, which coincides with the results in Fig. 2 (Fig. 3a, b).

In vitro stability of soluble transcription factors

The stability of transcription factors is important for effective gene regulation during cell culture. Here, we have investigated if 30Kc19 protein conjugation contributes to the stability of transcription factors. To test the effect of 30Kc19 protein on the stability of soluble transcription factors in solution, seven purified soluble transcription factors (Oct4-30Kc19, Sox2, Sox2-R9, Sox2-30Kc19, Klf4, Klf4-R9, and Klf4-30Kc19) were added and incubated in the culture medium. Oct4 alone and Oct4-R9 were ruled out as they were expressed as an inclusion body resulting in negligible amount of soluble proteins. In serum-free medium, 10–15 μg/ml of purified soluble transcription factors were incubated at 37 °C for 24 or 48 h before analysis. By using 30Kc19 protein as a fusion partner for transcription factors, we found that the stability of the soluble transcription factors was significantly increased (Fig. 4). The levels of Sox2 and Klf4 proteins produced alone or with R9 drastically decreased even within 24 h of incubation

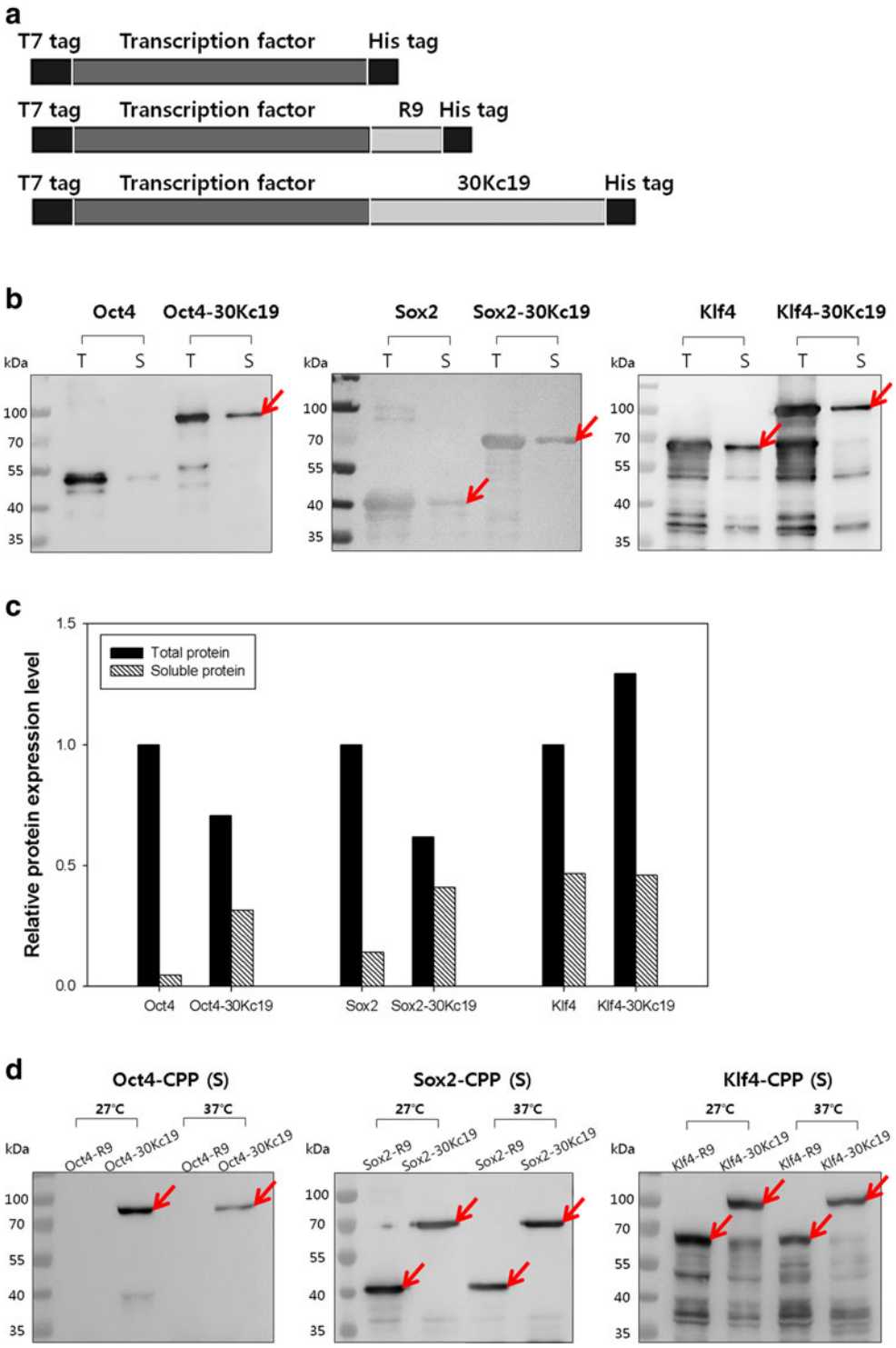

Fig. 2 Plasmid construction and soluble expression of transcription factors. **a** Transcription factors (Oct4, Sox2, c-Myc, Klf4, and L-Myc) were developed with and without nine-arginine (R9) or 30Kc19. **b** Comparison of soluble expression between transcription factors alone and transcription factors-30Kc19 at 37 °C. *E. coli* lysates were analyzed by Western blot. *T* total lysate, *S* soluble fraction. **c** The immunoreactive band intensities were quantified using ImageJ. **d** The effect of a CPP for solubilization of transcription factors at 27 and 37 °C. Soluble fractions were analyzed by Western blot. The *arrows* indicate soluble target proteins

in medium, indicating poor stability (Fig. 4b, c). In contrast, all the transcription factors conjugated with 30Kc19 protein were stable for 48 h of incubation. Thus, it is conclusive that the conjugation of 30Kc19 protein enhanced the stability of transcription factors in the medium.

Cell penetration, intracellular stability, and cytotoxicity of soluble transcription factors

In our previous work, 30Kc19 protein delivered cargo protein into various cells and organs with maintaining its stability and causing low cytotoxicity (Park et al.

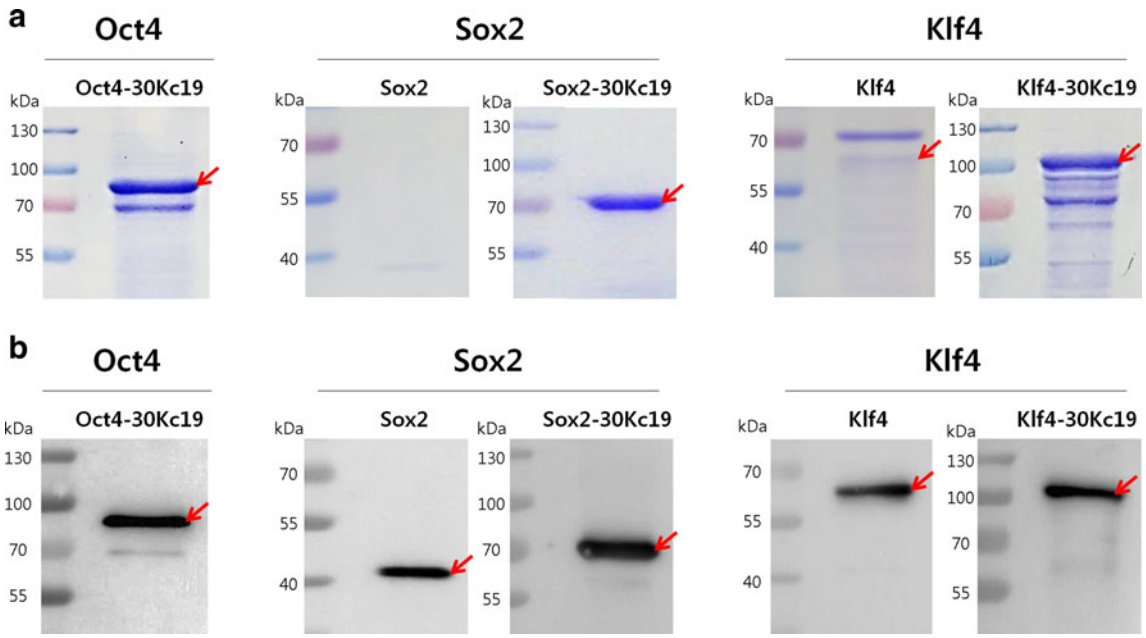

Fig. 3 SDS-PAGE and Western blot analysis of purified soluble transcription factors. Soluble transcription factors were purified using affinity chromatography. Purified proteins were separated by SDS-PAGE then visualized using Coommasie blue staining (**a**) or Western blot (**b**). The *arrows* indicate target proteins

2012a). Thus, the 30Kc19-conjugated transcription factors were selected for further study based on the following expectations: enhanced intracellular delivery and stability, and low cytotoxicity. First, the cell-penetration property of soluble transcription factors with 30Kc19 proteins was observed. To monitor the penetration of transcription factors into HDF cells, the proteins were labeled with Alexa Fluor® 488 and added to a culture medium at 20 μg/ml of the concentration. The fluorescence was observed in a live cell image using confocal microscopy. As shown in Fig. 5a, the transcription factor with the 30Kc19 protein was observed in the cells within 30 min of incubation, and its intracellular concentration gradually increased over time. This indicates that 30Kc19 protein conjugation successfully delivered transcription factors into cells.

To confirm how long 30Kc19-conjugated transcription factors remain in the cells, intracellular stability was observed by immunofluorescence analysis. We added 10 μg/ml of transcription factors to HDF cells

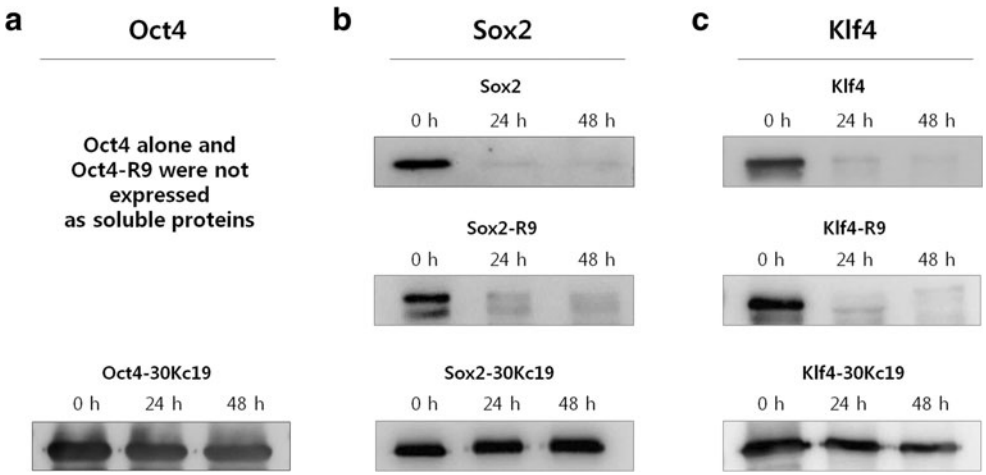

Fig. 4 In vitro stability of soluble transcription factors. **a** Purified Oct4-30Kc19 soluble protein was incubated in serum-free media at 37 °C and analyzed using Western blot. The results of Oct4 alone and Oct4-R9 are not shown as they were expressed as aggregate forms. **b**, **c** Purified Sox2 and Klf4 soluble protein were incubated with and without CPPs in serum-free media at 37 °C. After incubation for the indicated periods of time, the protein was analyzed by Western blot. Note that 30Kc19-conjugated transcription factors remained intact even at 48 h of incubation

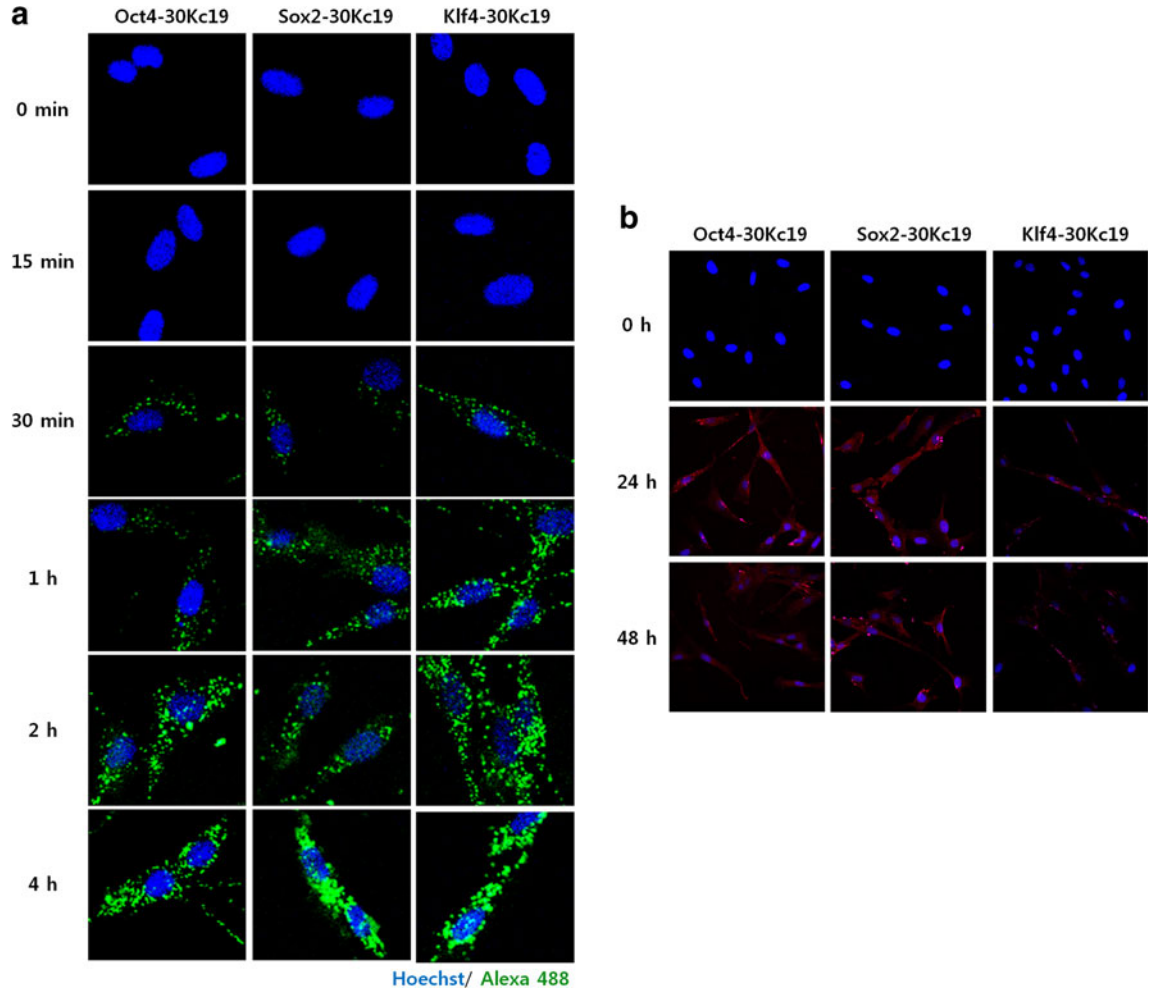

Fig. 5 Intracellular penetration and long-term stability of 30Kc19-conjugated soluble transcription factors. **a** Proteins were labeled with Alexa Fluor 488 (*green*) for live cell imaging of 30Kc19-conjugated transcription factors (Oct4, Sox2, and Klf4). HDF cells were treated with labeled proteins in a time-dependent manner and detected using confocal microscopy. **b** Intracellular proteins after incubation were analyzed by immunofluorescence. Alexa Fluor 594 (*red*) antibody for target proteins was used for the detection of transcription factors in the cells. Nuclei were visualized by Hoechst (*blue*)

for 24 and 48 h, respectively, and then the delivered proteins in the cells were detected with immunofluorescence. As shown in Fig. 5b, 30Kc19-conjugated transcription factors appeared to be stable inside the cells for up to 48 h. These results show that 30Kc19-conjugated transcription factors were stable in the cells and also agree with the in vitro results

Subsequently, we evaluated if 30Kc19-conjugated transcription factor has cytotoxicity. HDF cells were treated with the same concentration of four conjugated transcription factors simultaneously for 12 and 24 h in a dose-dependent manner, and then cell viabilities were assessed using MTT assay (Fig. S3). The concentration of each protein was 2, 5, 10, and 15 μg/ml, respectively. Even when the cells were treated with 60 μg/ml of four conjugated transcription factors (15 μg/ml each), there was no decrease in cell viability, meaning that 30Kc19-conjugated transcription factors have low cytotoxicity.

Transcriptional activity of 30Kc19-conjugated Klf4

Once introduced into cells, a transcription factor binds to its target DNA sequence in the nucleus for the control of transcription. Thus, we tested the transcriptional activity of a transcription factor-conjugated with 30Kc19 protein compared with that conjugated with R9. Among transcription factors, Klf4 protein was chosen to confirm whether our recombinant proteins have transcriptional activity in the cells. When cells were treated with Klf4-30Kc19 protein, luciferase activity increased significantly depending on the concentration (Fig. 6a). Then, the transcriptional activity of Klf4-conjugated with 30Kc19 or R9 was compared. One

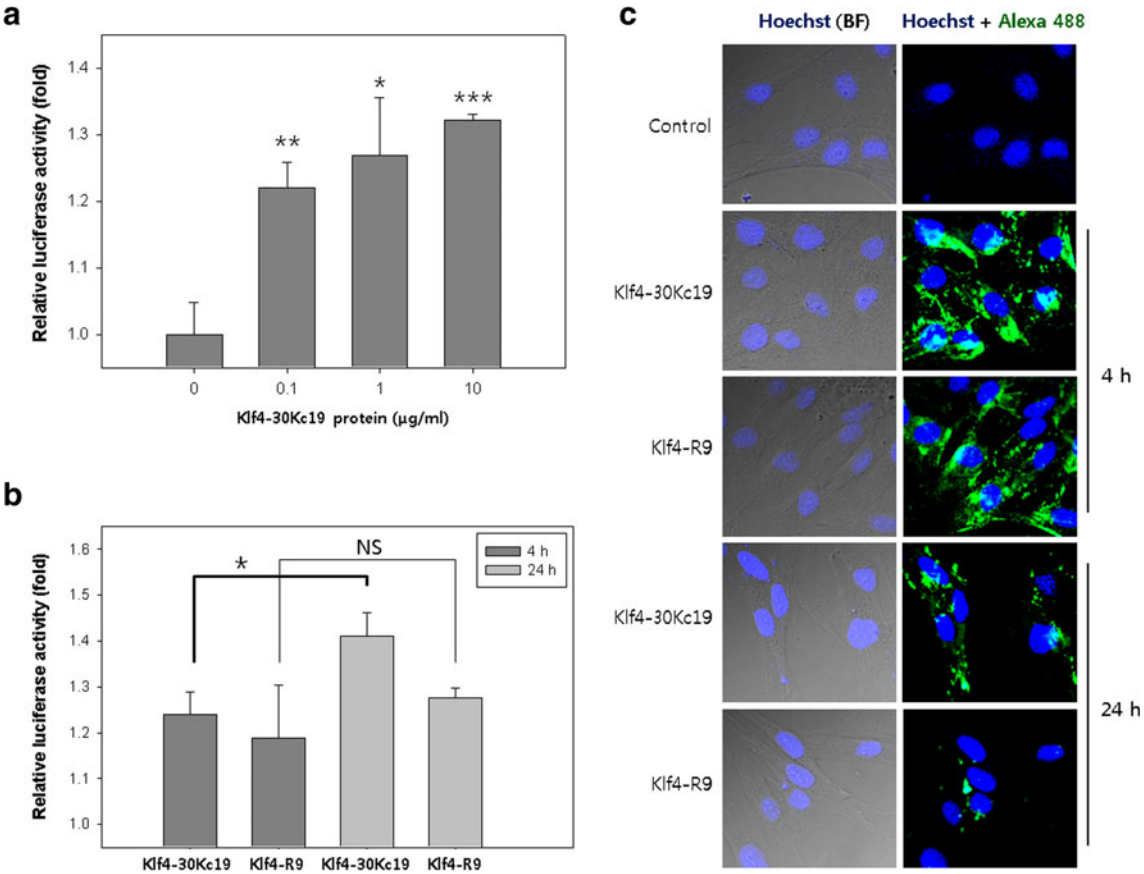

Fig. 6 Effect of 30Kc19 conjugation on intracellular transcriptional activity of Klf4 protein. **a** Klf4-30Kc19 protein was delivered into transfected cells, and luciferase activity was measured after 4 h to determine transcriptional activity. **b** One micrograms per milliliter of Klf4-R9 or Klf4-30Kc19 proteins were added to cells. Luciferase activity of Klf4 was measured after 4 or 24 h incubation, respectively (*$p<0.05$; **$p<0.005$; ***$p<0.0005$; *NS* nonsignificant). **c** Delivered Klf4 protein with 30Kc19 or R9 was analyzed by immunofluorescence. Green fluorescence represents the amount of Klf4 protein in the cells. Bright field (*BF*) images and fluorescence of nuclei (*blue*) images were merged and shown in the *left panel*. The *numbers* indicate the time of incubation

micrograms per milliliter of each transcription factor was delivered, and transcriptional activities were measured 4 and 24 h after delivery. Klf4-30Kc19 increased transcriptional activity ($p<0.05$) as time progressed (Fig. 6b). In contrast, there was no significant increase in transcriptional activity of Klf4-R9 (p=nonsignificant; NS). We can hypothesize that the higher transcriptional activity of Klf4-30Kc19 was due to the increased intracellular stability of Klf4 through 30Kc19 conjugation. Immunocytochemistry of intracellular Klf4-30Kc19 and Klf4-R9 showed that Klf4-R9 level in cells drastically decreased from 4 to 24 h after delivery (Fig. 6c). In contrast, there was a considerable amount of Klf4-30Kc19 still in the cells even at 24 h after delivery. This observation was similar to the in vitro stability results shown in Fig. 4. Thus, it is obvious that 30Kc19 conjugation enhanced transcriptional activity by increasing the intracellular stability of a transcription factor.

Discussion

In this study, 30Kc19 protein was applied as a fusion partner for the production of recombinant transcription factors that are used for the generation of iPSCs. It was hypothesized that the 30Kc19 protein has the potential to be developed as a fusion partner in the protein-based delivery of transcription factors for the regulation of gene expression, as it was shown to enhance the intracellular delivery as well as stability of its fusion protein. Interestingly, we have also found that the C-terminal fusion of the 30Kc19 protein promoted the soluble production of transcription factors, especially for Oct4 and L-Myc, while R9 did not. In fusion technology, fusion partners such as such as MBP, Trx, and NusA (Vincentelli et al. 2011) are usually added at the N-terminus to express soluble proteins (Sorensen and Mortensen 2005). However, because the N-terminus region of most transcription factors is important for their

functionality, in many cases, those typical fusion partners cannot be used. Unlike others, 30Kc19 protein can be placed at the C-terminus of target protein and increases solubility. Thus, the 30Kc19 protein is an eligible fusion partner for soluble expression of transcription factors.

Even though the recombinant proteins were produced as soluble forms, the extracellular and intracellular instabilities of these proteins after treatment are a critical problem still requiring a solution. It was reported that unstable soluble proteins are aggregated easily during cell culture (Sorensen and Mortensen 2005). There were trials to increase the stability of proteins by adding supplements such as lipid-rich albumin or serum (Thier et al. 2010; Thier et al. 2012). We observed that 30Kc19-conjugated transcription factors showed enhanced stability without additive materials to a culture medium. Previously, we reported that 30Kc19 protein increased enzyme stability via shielding effect; possibly by hydrophobic interaction (Park et al. 2012b). The increased stability of transcription factor is also considered to be due to the shielding effect of 30Kc19 protein. From luciferase and immunofluorescence assay, the results indicate that 30Kc19-conjugated with transcription factors enhances the intracellular stability of the protein and thus provides more opportunities for the transcription factor to further penetrate into the nucleus to carry out its role in binding to target DNA to participate in transcription. To accomplish successful protein-based reprogramming, a significant amount of proteins is required (Yang et al. 2009). However, because of a cytotoxicity hurdle, a small number of proteins (0.5–8 μg/ml) had to be added repeatedly, and 36 h was required between the cycles (Zhou et al. 2009). In the case of the 30Kc19-conjugated protein, even when the concentration of total proteins was 60 μg/ml, statistical differences in cytotoxicity were not observed. This demonstrates that 30Kc19 protein is a nontoxic carrier for the delivery of transcription factors and enhances that stability of adjacent proteins.

Protein-based delivery of transcription factors can be applied in many ways. Similar to iPSC generation, for direct lineage reprogramming that induces somatic cells into becoming a specialized cell type, defined transcription factors are required (Xu et al. 2015). Additionally, the nuclear receptors which are classified as transcription factors are potential drug candidates. These are related to metabolic diseases including cancer, infertility, obesity, and diabetes as potential drug candidates (Gronemeyer et al. 2004). In the use of transcription factors, protein can be an alternative method to avoid genomic integration that is possible with a conventional virus method. In this article, we have shown that a 30Kc19 cell-penetrating protein simultaneously enhances soluble expression, stability, and the transcriptional activity of transcription factors. Therefore, we propose that conjugation with the 30Kc19 protein is a novel approach that addresses the technical bottlenecks of gene regulation using transcription factors.

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Compliance with ethical standards

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Conflict of interest Author1 declares that she has no conflict of interest.
Author2 declares that he has no conflict of interest.
Author3 declares that he has no conflict of interest.
Author4 declares that he has no conflict of interest.
Author5 declares that he has no conflict of interest.
Author6 declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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