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TAT Kappa (TATK): A Novel Cell Penetrating Peptide for Delivery of Pluripotent Proteins into Target Cells

(TAT Kappa (TATK): Sel Baharu Menembusi Peptida untuk Penghantaran Protein Pluripoten kepada Sel Sasaran)

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ABSTRACT

Induced pluripotent stem cell (iPSC) holds a magnificent place in the medical revolution. Its emergence is expected to instigate development of novel therapies for regenerative medicine and treatment of malignant diseases. Moreover, iPSC usage also resolved a long-time ethical controversy on the usage of the embryo as a pluripotent stem cells source. Since Yamanaka's iPSC discovery in 2006, several pieces of research have proven that the enforced expression of transcription factors Oct-3/4, KLF4, and Sox2 can induce the reprogramming of previously differentiated cells, to generate iPSC. However, the conventional method using viral vectors leads to genetic modification due to exogene integration and subsequently tumorigenicity, which is unsafe for clinical application. Therefore, our study utilised an improved novel protein transduction domain, trans-activator of transcription kappa (TAT), a synthetic TAT-HIV to deliver these transcription factors gene as an alternative method for iPSC generation via non-viral reprogramming. With this new strategy, we have established a stable clone of 293T cells expressing TATκ fusion proteins (TATκ-GFP, TATκ-KLF4, TATκ-Sox2, and TATκ-Oct-3/4) that expresses and secretes their respective cloned reprogramming proteins. These stable clones successfully transduced our target cell (U937) monocyte cell line. TATκ-GFP, a marker protein and fusion proteins TATκ-KLF4, TATκ-Sox2, and TATκ-Oct-3/4 transduced the targeted (U937) monocyte cell line, proving that this novel TATκ possesses an ability to translocate across the cell membrane. Morphological changes were successfully observed in U937 cells after 20 days of transduction, however the presence of bonifide iPSC colonies were unable to be elicited. This might be due to the incomplete reprogramming or insufficient duration of protein transduction to generate iPSC cells.

Keywords: Cell-penetrating peptide; induced pluripotent stem cells; pluripotent proteins; reprogramming; transduction

ABSTRAK

Sel induk pluripoten terjana (iPSC) memainkan peranan penting dalam revolusi perubatan. Kemunculannya dijangka akan membuka ruang untuk pembangunan terapi baharu untuk perubatan regeneratif dan rawatan penyakit kanser malignan. Selain itu, penggunaan iPSC berupaya menyelesaikan kontroversi berhubung penggunaan embrio sebagai sumber sel induk pluripoten. Sejak penemuan iPSC pada tahun 2006 oleh Yamanaka, beberapa siri penyelidikan telah membuktikan bahawa pengekspresan gen secara paksa faktor transkripsi seperti Oct-3/4, KLF4 dan Sox2 berupaya memprogram-semula sel yang telah membeza untuk menghasilkan sel induk pluripoten. Namun, penggunaan kaedah konvensional vektor virus yang boleh mengubah genetik sel melalui integrasi atau penggabungan dengan eksogen berupaya mengakibatkan tumor dan ini menjadikan penggunaannya tidak selamat untuk aplikasi klinikal. Oleh itu, kajian ini menggunakan domain transduksi protein yang baharu dan diperbaharui yang dikenali sebagai trans-activator of transcription kappa (TATκ)*, iaitu TAT-HIV yang disintesis untuk menghantar gen faktor transkripsi sebagai kaedah alternatif penghasilan iPSC tanpa melalui proses program-semula menggunakan virus. Melalui kaedah baharu ini, klon stabil sel 293T yang merembeskan dan mengekspresikan protein program-semula tergabung TATκ (TATκ-GFP, TATκ-KLF4, TATκ-Sox2, dan TATκ-Oct-3/4) telah dihasilkan. Klon stabil ini telah berjaya mentransduksikan sel monosit sasaran (U937). TATκ-GFP yang memainkan peranan sebagai protein penanda serta protein tergabung TATκ-KLF4, TATκ-Sox2 dan TATκ-Oct-3/4 telah berjaya ditransduksikan ke dalam sel (U937), seterusnya membuktikan bahawa kaedah baharu yang mengaplikasikan TATκ ini berupaya untuk melalui proses translokasi merentasi membran sel. Perubahan morfologi sel U937 berjaya diperhatikan selepas 20 hari proses transduksi namun sel koloni iPSC tidak dapat dijana. Ini mungkin disebabkan oleh proses program-semula yang tidak lengkap atau tempoh transduksi yang tidak mencukupi untuk penjanaan sel iPSC.*

Kata kunci: Domain transduksi protein; faktor transkripsi; program-semula; sel induk pluripoten terjana; transduksi

Introduction

In 2006, Takahashi and Yamanaka first reported somatic cell reprogramming by induction with pluripotent maintaining transcription factors. Thereafter, a series of research has proven that enforced expression of pluripotency gene could reprogram any specialised cell into the induced pluripotent cell (iPSC) (Dimos et al. 2008; Kaji et al. 2009; Wernig et al. 2007; Yamanaka 2009). The conventional method of utilising a virus to integrate the pluripotent gene with the host gene results in exogene integration (Yamanaka 2009), making it unsuitable for clinical application (Dimos et al. 2008).

Various methods of genome non-modifying reprogramming have been studied in the past decade (Dimos et al. 2008; Kaji et al. 2009; Woltjen et al. 2009). Protein transduction technology (PTD) is one such technique that could deliver biomolecules using cell-penetrating peptides (CPPs) across the cell membrane without leaving a trace in the genome (Bechara & Sagan 2013; Deng et al. 2015). Historically, protein reprogramming started a decade ago after a mouse embryonic fibroblast (MEF) had been successfully reprogrammed using Oct4, Sox2, Klf4, and c-My reprogramming factors tagged with polyarginine (11R) cell-penetrating peptide (Zhou et al. 2009). Subsequently, a series of protein reprogramming techniques has generated stable iPSC cell lines efficiently (Kim et al. 2010; Nemes et al. 2014).

Furthermore, Zhang et al. (2012) have compared the efficiency of the Human Immunodeficiency Virus Transactivator of transcription protein domain (HIV-TAT) and polyarginine (11R), CPPs in cellular reprogramming. Reprogramming proteins fused with TAT was reported to be more transcriptionally active compared to 11R (Zhang et al. 2012). Nevertheless, protein reprogramming has not been well established due to its being less efficient and having a less defined transduction condition (Deng et al. 2015). For this reason, CPPs are constantly modified to increase their transduction efficiency. Therefore, this current study aimed to utilise a novel cell-penetrating peptide, i.e., TATκ, a derivative from HIV-TAT.

HIV-TAT is a protein sequence that regulates long terminal repeats (LTR) promoter for viral production (Frankel & Pabo 1988: Green & Loewenstein 1988; Rana & Jeang 1999) discovered the ability of TAT in penetrating the cell membrane. Following this, a wild-type TAT was mutated by Green and Loewenstein (1988) and the mutated form of CPP was reported to have enhanced the transduction process. Thenceforth, HIV-TAT peptide has been proven to enhance the transduction capability (Denault & Leduc 1996; Mi et al. 2000).

Apparently, in this current study, TATκ (YARKAARQARA) was utilised, which had been created by destroying two Furin cleavage sites that normally exist in wild-type TAT peptide. Furin is a paired basic amino acid cleaving enzyme (PACE) that exists in the cytoplasm, predominantly in the Golgi apparatus (Denault & Leduc 1996). Due to the presence of Furin cleavage site, the TAT would be cleaved from the fusion protein thus, inhibiting the transduction and translocation of the fusion protein into the target cells (Tikhonov et al. 2004).

To overcome this problem, amino acid sequences RQRR and RKKR of wild-type TAT were altered by replacing five Ala residues within the peptide (Figure 1), named as TATκ. TATκ integrated the Schematic diagram of pSecTag2B circular vector. TATκ tagged the green fluorescent protein (GFP), and a protein with tumour-specific cytotoxicity successfully retained its functionality in translocated cells (Flinterman et al. 2009). Therefore, this current study aimed to utilise this novel CPP (i.e. TATκ) to deliver the transcription factors for reprogramming. A strategy was proposed to generate a stable cell line that would secrete reprogramming proteins to carry TATκ and mediate the delivery. This study also aimed to validate the translocation and reprogramming efficiency of the modified novel cell-penetrating peptide TATκ in tagging pluripotent proteins for the first time.

Materials and Methods

Cell lines

Human embryo kidney (HEK) cells, which had been transformed with T-antigen 293T, were cultured in commercially available DMEM (Dulbecco's Modified Eagle Medium, Gibco Thermofisher Scientific, USA), completed with 10% (v/v) heat-inactivated, foetal bovine serum (FBS) (Gibco Thermofisher Scientific, USA), and 100 µg/mL penicillin-streptomycin (Gibco Thermofisher Scientific, USA). Meanwhile, the U937 (human lymphocyte) cell line was cultured in RPMI 1640 (Roswell Park Memorial Institute, Gibco Thermofisher Scientific), completed with 10% (v/v) heat-inactivated, foetal bovine serum (FBS) (Gibco Thermofisher Scientific, USA), and 100 µg/mL penicillin-streptomycin (Gibco Thermofisher Scientific, USA).

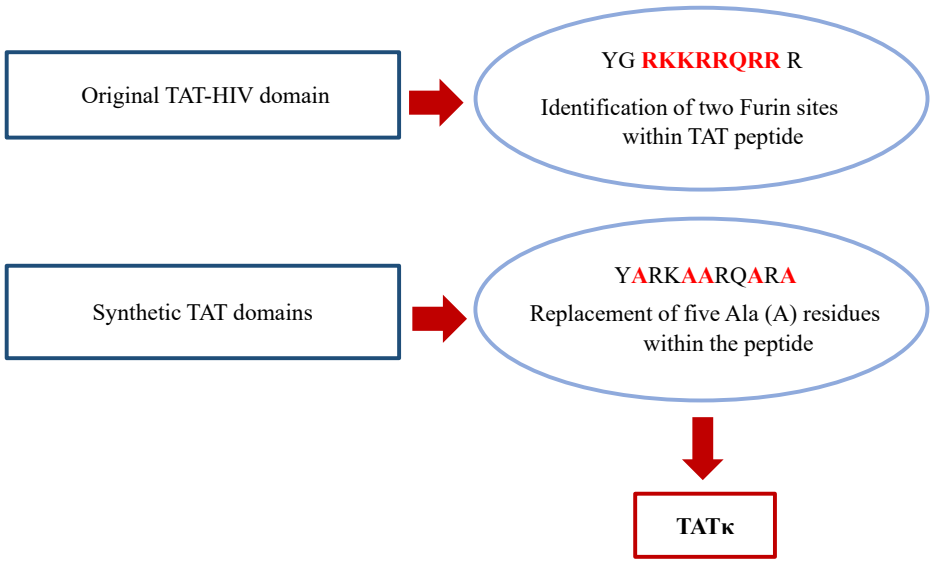

FIGURE 1. Illustration of TATκ synthesis from the original TAT

TRANSIENT TRANSFECTION

A standard conventional calcium phosphate (Ca-PO4) co-precipitation transfection protocol was followed (Kingston et al. 2003). Concisely, 1 × 10^6 293T cells were seeded in 100 mm diameter treated culture dishes (Falcon). On the following day, a mixture of DNA and calcium phosphate co-precipitation was prepared by mixing 20 µg of TATκ-GFP, TATκ-KLF4, TATκ-Sox2, and TATκ-Oct-3/4 per culture plate, 0.5 M, and 2. HEBS (HEPES Buffered Saline) at pH 6.7 and was left to stand for 30 min at room temperature. Subsequently, the mixture was added dropwise to the culture dish containing 293T cells. Following 72 h of transfection, TATκ-GFP expressing cells could be visualised through a fluorescent microscope and were harvested to determine their expression by flow cytometer (FACSVerse™, BD Biosciences USA). Meanwhile, TATκGFP, TATκ-KLF4, TATκ-Sox2, and TATκ-Oct-3/4 pluripotent proteins expressing cells and conditioned media were harvested to detect their expression and secretion by western blot analysis. Experiment conducted in triplicates.

STABLE TRANSFECTION

The same protocol as transient transfection was used for the establishment of stable TATκ fusion proteins producing cell lines. Briefly, 72 h post-transfection, 2.5 µg/mL of puromycin (Sigma Aldrich, USA) was added to the culture media. Plasmid integrated cells expressed the puromycin resistance gene and survived in the selection media. The selection media with puromycin was changed every 3-4 days until the cells formed a single colony. Subsequently, the single colony cells were collected using a sterile pipette and transferred to 6-well plates with the selection media containing puromycin. Cells were allowed to reach confluency prior to harvest, and the conditioned media was concentrated for the western blot analysis, to monitor protein expression and secretion. Upon confirmation, stable clones were cryopreserved. TATκ-GFP expressing 3 stable clones were harvested for FACS analysis.

WESTERN BLOT ANALYSIS

For adherent cell lysates, cells were washed twice with cold DPBS, and 500 µL of RIPA buffer (Thermofisher, USA) were added to the culture dish. Immediately, the plate was put on ice for 5 min and occasionally swirled for uniform dispersion. The lysates were collected using a cell scraper. Subsequent to this, the lysates were centrifuged at ~14000 g for 15 min to pellet the debris, and the supernatant was stored at -20 °C. For suspension cell lysates, cells were pelleted down by centrifugation at 2500 g for 5 min and the pellet was washed twice in cold DPBS, and again pelleted down by centrifugation at 2500 g for 5 min. Next, RIPA buffer was

added (100 µL for 1 × 10^6 cells) and shaken gently for 15 min on ice. The lysates were centrifuged at ~14000 g for 15 min to pellet the debris, and the supernatant was stored at -20 °C.

For the preparation of the culture medium containing secreted proteins, the conditioned medium was concentrated using Vivaspin spin column (GE Healthcare, USA) at ~3900 g for 30 min at 4 °C. The concentrated medium was stored at -20 °C for further analysis. The protein samples were quantified using the Pierce BCA Protein Assay Kit (Thermofisher, USA). Protein samples (10 µg/mL) were mixed with 2× LSB Buffer, and were boiled at 95 °C for 5-10 min. The proteins samples were then separated using 10% SDS-polyacrylamide gels (SDS-PAGE), and were electrophoretically transferred to nitrocellulose membranes (Invitrogen, GE Healthcare) via a wet transfer system (Invitrogen, USA). The protein-transferred membranes were blocked in a blocking buffer containing 1×TBS, 0.1% Tween 20 (Sigma Aldrich, USA), and 5% (w/v) semi-skimmed milk powder (Sigma Aldrich, USA) for 1 h.

Next, the membranes were washed three times in a washing buffer containing TBS-Tween 20 for 15 min. The following antibodies were diluted (1:1000) in an antibody buffer containing 1×TBS, 0.1% Tween 20, 1% Bovine Serum Albumin (BSA) (Sigma Aldrich, USA), and were incubated overnight at 4 °C. Primary antibodies involved were: anti-GFP (D5.1) XP® Rabbit mAb (Cell Signalling, USA); KLF4 (D1F2) Rabbit mAb (Cell Signalling, USA); Sox2 (D6D9) XP® Rabbit mAb (Cell Signalling, USA); Oct-3/4 primary antibody Rabbit mAb (R&D System, USA); α-tubulin Rabbit mAb (Cell Signalling, USA), Anti-Rabbit IgG, HRP-linked antibody (Cell Signalling, USA); Human oesophagus cell lysate (positive control, Santa Cruz, USA); Raji Cell lysate (Santa Cruz, USA); and Hela cell lysate (in-house production). Lastly, the immunoreactive bands were detected by enhanced chemiluminescence (ECL plus; GE Healthcare) and bands could be visualised via the C-DiGit™ Blot Scanner (LI-COR Biosciences). Western blotting analysis carried out 3 times for each validation of protein expression and secretion.

TRANSDUCTION OF TARGET CELLS

A total of 1×10^5 293T cells (wild-type as negative control), and TATκ fusion protein-producing stable cell lines, were seeded in 6-well plates and grown to 50-70% confluence. A total of 1×10^5 target cells (U937) were seeded in Millicell ® Cell Culture Insert (Merck, USA) Transwell insert. Subsequently, valproic acid (VPA) with a final concentration of 1 mM, as a histone deacetylase inhibitor (epigenetic modifier), was added to enhance iPSC reprogramming. VPA would mask the target cells with a negative charge and would increase the uptake of positively charged TATκ fusion from stable producer cells. Both cells were co-cultured together for 40 days. Both the producer (293T) and target (U937) cells were lysed, as mentioned earlier, after the 3rd day and 14th day of transduction to detect TATκ fused pluripotent proteins in the U937 cells. Cells from 3 independent experiments subjected to western blotting analysis. Morphological changes of cells captured by light microscope.

RESULTS AND DISCUSSION

ESTABLISHMENT OF TATκ-GFP, TATκ-KLF4, TATκ-Sox2, AND TATκ-Oct-3/4 SECRETING STABLE PRODUCER CELL LINE

Marker protein TATκ-GFP was observable through fluorescent microscopy (Figure 2), while flow cytometry confirmed the positive population of TATκ-GFP (Figure 3). Subsequently, western blot analysis confirmed the expression and secretion of TATκ-GFP, TATκ-KLF4, TATκ-Sox2, and TATκ-Oct-3/4 of the stable producer cell line (Figure 4).

TRANSDUCTION OF TATκ-GFP, TATκ-KLF4, TATκ-Sox2, AND TATκ-Oct-3/4 PROTEINS

A density of 10000 cells/cm^2 of target cell (U937) hematopoietic cell line was transduced with TATκ-GFP, TATκ-KLF4, TATκ-Sox2, and TATκ-Oct-3/4 proteins secreted from producer cells via co-culture method. 293T (producer cell) lysate and U937 (target cells) were lysed and subjected to the western blot analysis to confirm the secretion and uptake of proteins (Figure 5). Transduction of proteins was monitored on the 3rd and 14th day of the co-culture of producer and the target cells.

MORPHOLOGY CHANGES OF TATκ PLURIPOTENT PROTEINS TRANSDUCED TARGET CELLS (U937)

Morphological changes of the target cells (U937) from direct co-culture systems could not be seen clearly since both the producer and the target cells were not separated using Transwell. However, U937 cells from the 'indirect' co-culture system showed morphological changes 20 days post-transduction (Figure 6). The U937 cells elongated and started to adhere to the surface. However, the cells did not form iPSC colony-like structure even after 40 days of transduction (Figure 6).

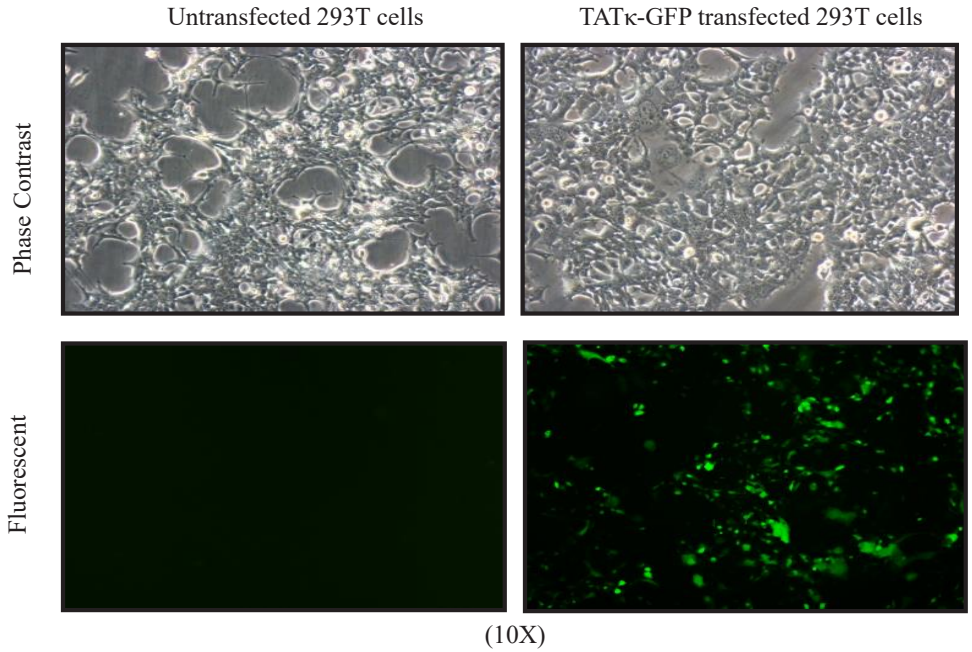

FIGURE 2. Fluorescent microscope analysis of TATκ-GFP transfected and untransfected 293T cells. *All pictures have taken at 10× magnification, *Results shown is representative of technical replicates

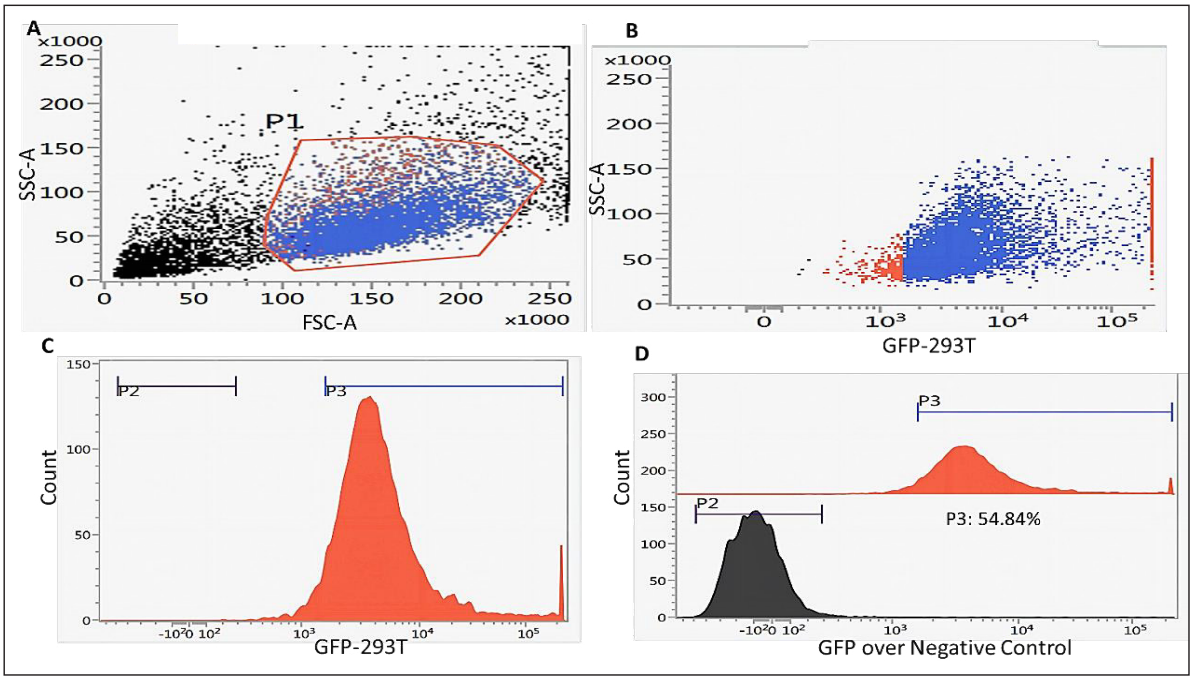

FIGURE 3. FACS analysis of transient TATκ-GFP expression. (A) dot plot of GFP expressing 293T cells (B) GFP positive population of 293T expressing TATκ-GFP, (C) histogram of GFP expression, (D) Histogram overlay of GFP expression (54.84%) and negative control, *Results shown is representative of technical replicates

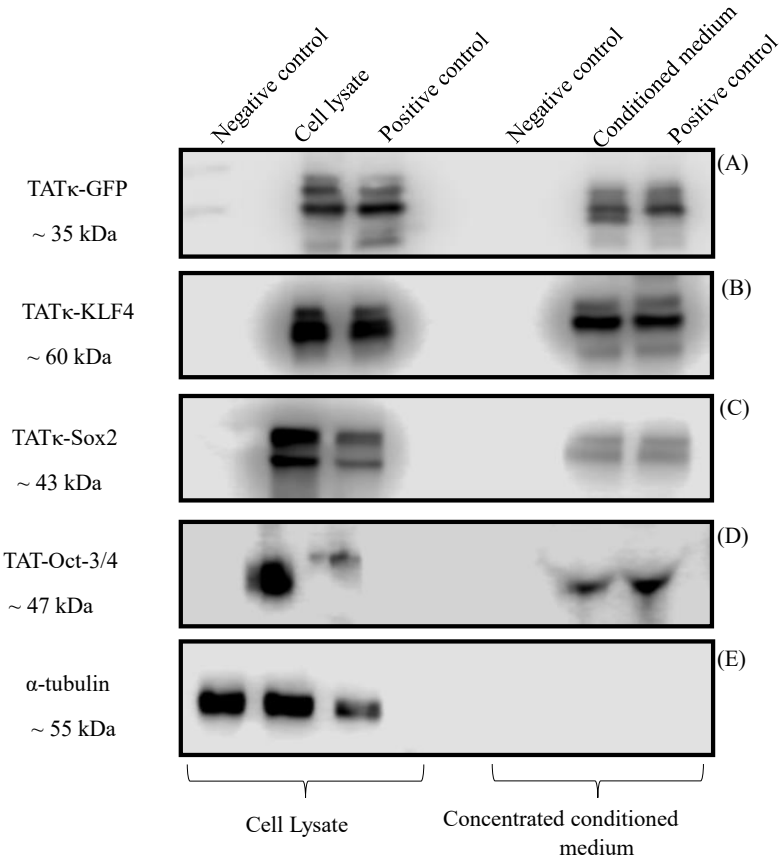

FIGURE 4. Western Blotting Analysis, (A) Expression and secretion of TATκ-GFP; (B) Expression and secretion of TATκ-KLF4; (C) Expression and secretion of TATκ-Sox2; (D) Expression and secretion of TAT-κ-Oct-3/4, (E) α-tubulin as the loading control for all cell lysates. Untransfected 293T cell lysate and conditioned media as the negative control. Previously confirmed the expression of respective proteins which were used as the positive control for all, *Results shown is representative of technical replicates

This current study has successfully expanded the expression vector pSecTag2B that carries the TATκ transduction domain tagged with GFP and pluripotent factors (KLF4, Sox2, and Oct-3/4). Using these plasmids, stable clones were established, expressing TATκ-GFP, TATκ-KLF4, TATκ-Sox2, and TATκ-Oct-3/4 proteins, which were validated by western blot analysis. Similarly, Kim et al. (2010) employed a similar strategy, by establishing 293T stable cell line secreting pluripotent factors tagged with 9R (arginine cell-penetrating peptide), and subsequently reprogrammed the cells using the cell extract. Likewise, 293T stable mixed population cells expressing TATκ-Oct-3/4 and TATκ-KLF4 were established in another previous study (Nordin et al. 2014). Other independent studies generated iPSCs from the continuous transduction of pluripotent proteins, which fused TAT Cell-penetrating peptide (Nemes et al. 2014; Zhang et al. 2012). The pluripotent proteins were scaled up using a bacterial system and the concentration, as well as the incubation period for the reprogramming, was optimised. According to these studies, the protein transduction process must be in a continuous manner to sustain the activity of the protein to reprogram cells. To overcome this limitation, this current study generated a stable cell line to be co-cultured with the target cells, so that the constant supply and activity of pluripotent proteins can be achieved.

Furthermore, the previous studies on iPSCs reprogramming using protein transduction have optimised the protein concentration and incubation period. However, the optimisation varies according to the use of CPP (Kim et al. 2010; Nemes et al. 2014) hence, it is necessary to optimise the conditions again upon using different CPP. Therefore, this current study employed

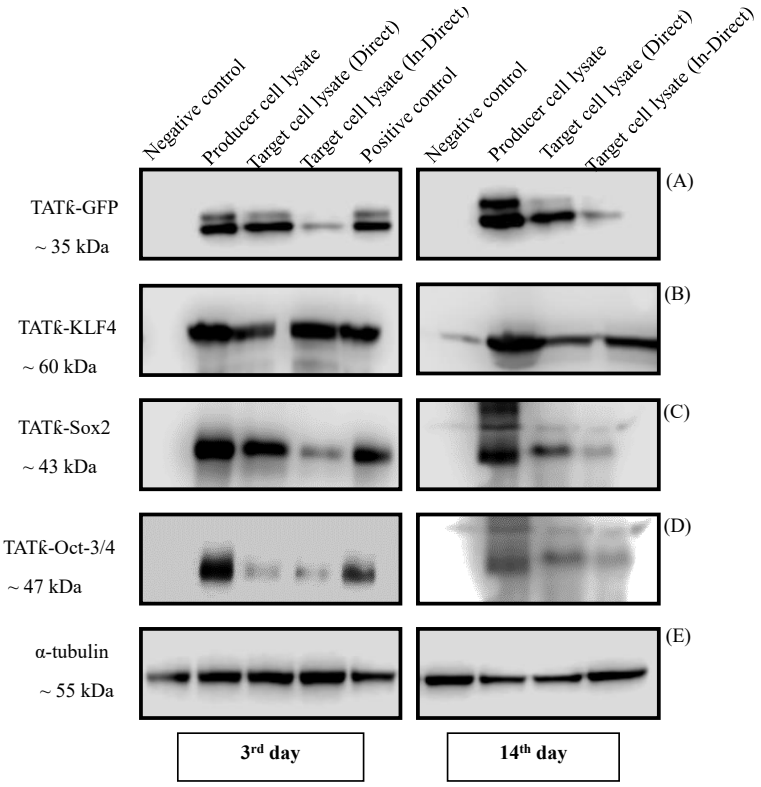

FIGURE 5. Western Blotting Analysis, (A) Expression of TATκ-GFP in producer cell (293T), Transduction of TATκ-GFP in target cell (U937); (B) Expression of TATκ-KLF4 in producer cell (293T), Transduction of TATκ-KLF4 in target cell (u937), (C) Expression of TATκ-KSox2 in producer cell (293T), Transduction of TATκ-Sox2 in target cell (U937), (D) Expression of TATκ-Oct-3/4 in producer cell (293T), Transduction of TATκ-Oct-3/4 in target cell (U937). Untransduced U937 cell lysate was used as the negative control for 293T lysate, and previously confirmed the expression of respective proteins which were used as the positive control for all; (E) α-tubulin act as the loading control for all, *Results shown is representative of technical replicates

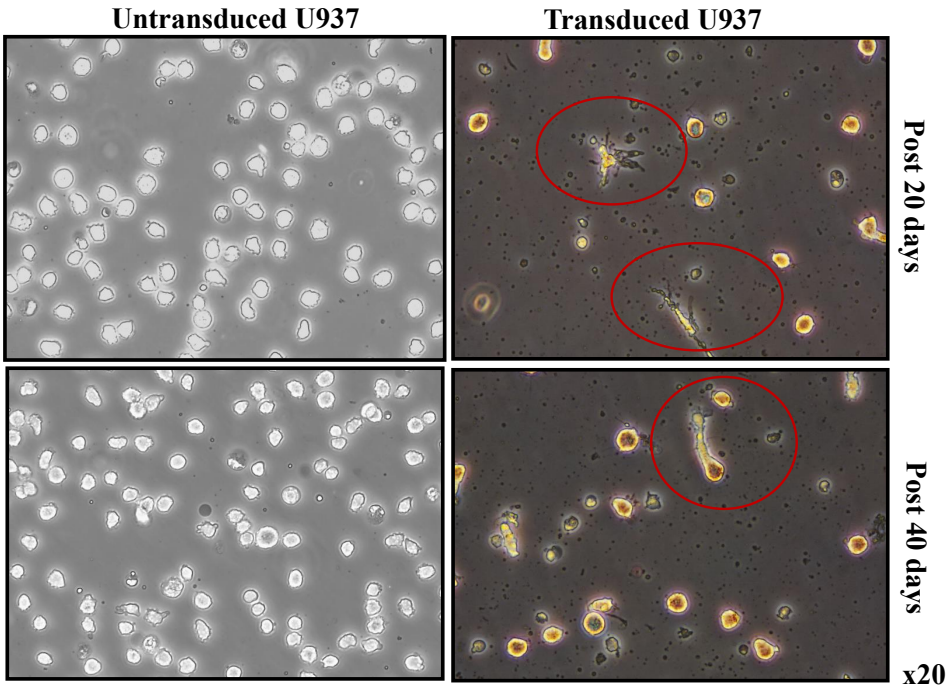

FIGURE 6. Morphology changes of the target cells (U937) post 20 and 40 days of protein transduction (indirect transduction). *All pictures have taken at ×10 magnification, *Results shown is representative of technical replicates

a co-culture method to avoid the necessity of protein purification and transduction condition optimisation. Two types of co-culture methods were carried out, namely 'direct' and 'indirect'. In the 'direct' co-culture experiment setting, both the producer and the target cells were co-cultured together, which allowed a direct cell to cell contact.

Meanwhile, in the 'indirect' co-culture experiment setting, the producer and the target cells were separated using a 0.45 μm Transwell, which acted as a barrier between both cells. The cells were separated because culturing two types of cells together might restrict their usage in future clinical application. Moreover, the 'direct' method posed a risk in the harvesting of the producer cells, which might have given a false-positive result when detecting the reprogramming protein translocation in the target cells. Notwithstanding, the 'direct' co-culture method was experimented in this study, since the technique would be able to provide full exposure to the protein secreted by the producer, which might increase the efficiency of uptake. To ensure co-culture system in this experiment would supply the protein continuously, the U937 cells were lysed on the 3rd day and 14th day post co-culture.

The transduction of reprogramming proteins was detected using the western blot analysis. This was to ensure the presence of proteins up to 14 days, because the iPSC colony would start to appear after 14 days of transduction in accordance with previous research (Zhang et al. 2012). The western blot analysis on U937 confirmed the presence of all reprogramming proteins in the target cells, which confirmed the transduction or penetration potential of novel TATκ as a cell-penetrating peptide. However, the 'direct' co-culture system was discontinued after difficulties had arisen in observing the morphology changes of the target cells (U937) due to the interference of adherent 293T producer cells. Unlikely, morphology changes could be observed in U937 cells, which were grown in Transwell, inserted together with the producer cells seeded at the bottom of the well. Transduced U937 cells started to elongate and adhere post 20 days of transduction, as shown in Figure 6.

Unfortunately, iPSC-like colonies did not form and the cells remained elongated post 40 days of transduction. It can be postulated that a non-treated surface, like the transwell insert, is not suitable for iPSC adherence. Practically, iPSC colonies require a matrix-coated surface to attach and expand (Jiang et al. 2019; Xu et al. 2001). Nevertheless, commercially available coating could not be obtained for this study, which might have disrupted the passage of reprogramming proteins from the producer cells that seeded on the bottom of the well. Elongation of U937 from its intrinsic monocyte morphology might be due to partial reprogramming. One of the hypothesised reasons is that the protein secreted by the producer cells might not be adequate to induce iPSC reprogramming. Therefore, protein quantification needs to be done in the future to optimise a more defined transduction condition. Besides this, the three types of reprogramming proteins alone (TATκ-KLF4, TATκ-Sox2, and TATκ-Oct-3/4) might not be sufficient to induce reprogramming. Correspondingly, Zhang et al. (2012) reported a failure to generate iPSC-like colonies using four factors (Oct-3/4, KLF4, Sox2, and c-Myc) tagged TAT, while colonies appeared upon the addition of Nanog to the list. This current study excluded c-Myc due to its tumorigenicity activation potential. Nakagawa et al. (2008) also generated iPSC successfully by excluding c-Myc and by using retroviral transduction (Okita et al. 2007).

On the other hand, other studies on protein reprogramming included c-Myc as one of the factors (Kim et al. 2010; Nemes et al. 2014; Zhang et al. 2012; Zhou et al. 2009). Therefore, in future studies, c-Myc can be included as one of the factors to generate iPSC. In addition, the transduction period might not be adequate to generate iPSC. Nemes et al. (2014) observed iPSC colonies after 40 days of transduction, while Kim et al. (2010) generated iPSC after 35 days of transduction. In this current study, the transduction was discontinued after 40 days, since no iPSC colony had formed. By prolonging the transduction period in future studies, the iPSC colony may be obtained.

LIMITATIONS AND RECOMMENDATIONS

This study confirmed the transduction affinity of the novel TATκ protein transduction domain to deliver pluripotent factors. However, the co-culture method could not reprogram U937 cells to iPSC. Therefore, purifying the TATκ fused reprogramming proteins will be a better option to optimise the concentration and protein incubation period, to set up a more defined protein transduction condition. Moreover, the untreated surface of the Transwell insert has become an unsuitable candidate in generating iPSC colonies. Elongated and adhered U937 cells could not grab the surface and were detached; this restricted the researchers from conducting iPSC characterisation staining, like alkaline phosphatase, to characterise reprogrammed cells. Moreover, Nanog and c-Myc could be cloned to the TATκ sequence to establish a stable cell line to transduce the target cells.

Conclusion

This current study generated 293T stable cell lines that secreted novel cell-penetrating peptide TATκ, fused with Sox2, KLF4, and Oct-3/4 pluripotent proteins. These proteins successfully penetrated the U937 monocyte cell line. The U937 cells elongated and adhered post 20 days of protein transduction, but did not form iPSC colonies, so the transduction was discontinued on day 40.

Acknowledgements

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