Stem Cell Research 25 (2017) 88–97

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Enhanced human somatic cell reprogramming efficiency by fusion of the MYC transactivation domain and OCT4

Ling Wang [a,1], Delun Huang [a,b,1], Chang Huang [a], Yexuan Yin [a], Kaneha Vali [a], Ming Zhang [b], Young Tang [a,*]

[a] Department of Animal Science, Institute for Systems Genomics, University of Connecticut, Storrs, CT, USA
[b] State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Animal Reproduction Institute, Guangxi University, Nanning, Guangxi, China

ARTICLE INFO

Article history:
Received 2 August 2017
Received in revised form 20 October 2017
Accepted 23 October 2017
Available online 26 October 2017

Keywords:
OCT4
MYC
Fusion protein
Reprogramming
Transactivation domain
Induced pluripotent stem cells

ABSTRACT

The development of human induced pluripotent stem cells (iPSCs) holds great promise for regenerative medicine. However the iPSC induction efficiency is still very low and with lengthy reprogramming process. We utilized the highly potent transactivation domain (TAD) of MYC protein to engineer the human OCT4 fusion proteins. Applying the MYC-TAD-OCT4 fusion proteins in mouse iPSC generation leads to shorter reprogramming dynamics, with earlier activation of pluripotent markers in reprogrammed cells than wild type OCT4 (wt-OCT4). Dramatic enhancement of iPSC colony induction efficiency and shortened reprogramming dynamics were observed when these MYC-TAD-OCT4 fusion proteins were used to reprogram primary human cells. The OCT4 fusion proteins induced human iPSCs are pluripotent. We further show that the MYC Box I (MBI) is dispensable while both MBII and the linking region between MBI/II are essential for the enhanced reprogramming activity of MYC-TAD-OCT4 fusion protein. Consistent with an enhanced transcription activity, the engineered OCT4 significantly stimulated the expression of genes specifically targeted by OCT4-alone, OCT4/SOX2, and OCT4/SOX2/KLF4 during human iPSC induction, compared with the wt-OCT4. The MYC-TAD-OCT4 fusion proteins we generated will be valuable tools for studying the reprogramming mechanisms and for efficient iPSC generation for humans as well as for other species.

1. Introduction

The induced pluripotent stem cell (iPSC) technology has revolutionized the regenerative medicine research since first reported a decade ago (Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Yu et al., 2007), by establishing the equivalency of embryonic stem cells (ESCs) while avoiding the controversial embryo destruction. Derivation of patient-specific iPSCs and directed differentiation into specific cell types or tissues combined with the recent development in gene-editing technology provided new avenues for disease modeling, drug screening, and gene-therapy research (Clevers, 2016; Passier et al., 2016; Yamanaka, 2007). Numerous efforts were taken to improve the efficiency of iPSC induction from somatic cells. These include using various starting cell types, different transgene delivery methods, chemical additives, and extra reprogramming factors in addition to the "OKSM" (OCT4, KLF4, SOX2, and c-MYC) Yamanaka factors (Ebrahimi, 2015; Malik and Rao, 2013). However, although various methods are available to overexpress OKSM in somatic cells, the reprogramming efficiency for human iPSC generation is generally at the lower end of the reported range (0.00002 - ~1%) and varies among different laboratories (Malik and Rao, 2013; Rao and Malik, 2012). Also, it usually takes 3 to 5 weeks for the induced iPSC colonies to appear (Malik and Rao, 2013; Rao and Malik, 2012). The low efficiency and lengthy duration of iPSC generation represent a hurdle for robust induction of iPSCs in research laboratories to further advance the discovery, development, and test of new therapeutics using this technology.

One promising strategy to facilitate reprogramming is to enhance the functionality of the OKSM reprogramming factors using protein engineering. A greater transcription activity has been reported by fusing the transactivation domain (TAD) of MyoD or the herpes simplex virus protein VP16 (MyoD-TAD or VP16-TAD) with the reprogramming factors, thus enhances the reprogramming efficiency and shortens the dynamics of iPSC induction (Hirai et al., 2011; Wang et al., 2011). The MyoD-TAD is potent at remodeling and relaxing the chromatins through recruitment of histone modifiers PCAF, p300/CBP, and SWI/

Abbreviations: AP, Alkaline Phosphatase; EB, Embryoid Body; ESC, embryonic stem cell; DBD, DNA-binding domain; HNFK, human neonatal foreskin keratinocyte; HUC-MSC, Human umbilical cord-derived mesenchymal stem cell; iPSC, induced pluripotent stem cell; KMS, KLF4, MYC, and SOX2; MBI/II, MYC box I/II; MET, mesenchymal to epithelial transition; MSC, mesenchymal stem cell; MYC-TAD-OCT4, MYC-TAD fusion to OCT4 Protein; NLS, nuclear localization signal; OG-MEF, mouse embryonic fibroblasts with GFP expression cassette driven by the Oct4 promoter; OKSM, OCT4, KLF4, SOX2, and c-MYC; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; TAD, transactivation domain; wt-OCT4, wild type OCT4.

* Corresponding author.
E-mail address: yong.tang@uconn.edu (Y. Tang).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.scr.2017.10.014

SNF chromatin-remodeling complexes to target genes. The VP16-TAD activates gene expression through interaction with basal transcription factors as well as chromatin-remodeling complexes (Hirai et al., 2010). While the MyoD- and VP16-TAD could promote reprogramming, the intention to increase the reprogramming activity of SOX2 and KLF4 using MyoD-TAD fusion was unsuccessful (Hirai et al., 2011). Also, as a viral protein, the impact of VP16-TAD to induced iPSCs may need more characterization.

c-MYC (MYC) is one of the original Yamanaka factors, and greatly promotes the efficiency of iPSC colony formation together with the other three reprogramming factors (Takahashi and Yamanaka, 2006). MYC-TAD can be a powerful candidate for protein fusion since it is known to activate gene expression at multiple levels. These include 1) promoting transcription initiation through interaction with basal transcription factors (McEwan et al., 1996), 2) facilitating chromatin accessibility through recruitment of histone acetyltransferase complexes like TRRAP (McMahon et al., 1998), p300/CBP, and Gcn5 (Flinn et al., 2002; Zhang et al., 2014), and 3) enhancement of translation through increasing mRNA cap methylation, polysome loading, and phosphorylation of the RNA Polymerase II carboxy terminal domain (Cowling and Cole, 2007). In fact, in yeast, MYC-TAD was shown to stimulate strong gene expression when fused to the GAL4 protein DNA binding domain (DBD) (Kato et al., 1990). Also, the bacterial DNA binding protein LexA fusion with MYC-TAD exhibited comparable or even greater transactivation activity in yeast when compared with the fusion with VP16-TAD (Salghetti et al., 2001). We therefore hypothesized that the reprogramming activity of Yamanaka factors could be dramatically enhanced by fusion with MYC-TAD.

In this study, we fuse MYC-TAD to the N-terminus (N-term) of human OCT4, and defined the fraction of MYC-TAD required for enhancing reprogramming. We show that the fusion of MYC-TAD to OCT4 can shorten the reprogramming dynamics in mouse and human cells, and dramatically enhance the reprogramming efficiency for human iPSC induction.

2. Materials and methods

2.1. Reprogramming constructs and viral preparation

Human reprogramming factors OCT4, SOX2, KLF4 and MYC were used in this project for both mouse and human somatic cell reprogramming. Different MYC DNA fragments were PCR amplified from pMXs-c-MYC plasmid (Addgene, Cambridge, MA) and fused to the 5′ end of wild type OCT4 (Table S1). The MYC and OCT4 DNA fragments were joined into pMXs retroviral vector (Cell Biolabs, Inc., San Diege, CA) using an in-fusion kit (Clontech, Mountain View, CA). Polycistronic DNA encoding KLF4, MYC and SOX2 were re-cloned from the 4F2A lentiviral vector (Addgene) into the pMXs vector (Table S1). For retroviral packaging, pMXs constructs were co-transfected into 293T cells with PUMVC and pCMV-VSV-G plasmids (Addgene) using Fugene 6 (Promega, Madison, WI) according to the protocol from Addgene website. Virus-containing supernatant was harvested 48 h and 72 h post-transfection and filtered through 0.8 μm filters. Viral aliquots were stored at −70 °C before use.

2.2. Generation of mouse iPSCs

Mouse embryonic fibroblasts (OG-MEFs) were generated from E13.5 embryos as described previously (Tang et al., 2011). Reprogramming assays were carried out based on our previous protocol (Tang et al., 2011) with some minor adaptations. OG-MEFs at passage (P) 3–5 were seeded at 3.5×10^5 cells per 6-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. The next day (day 0), MEFs were incubated with virus-containing medium for around 20 h with 10 μg/ml polybrene (American Bioanalytical, Natick, MA). Three days (day 3) after infection, the medium was changed to mouse reprogramming medium as described below. On day 5, transduced MEFs were trypsinized and passaged onto mitomycin C treated CF-1 feeder cells (GlobalStem, Rockville, MD). Colony formation and GFP fluorescence appearance was scored starting from day 10 under a Nikon fluorescent microscope. The reprogramming medium was a 1:1 mix of ESC medium containing 20% knock-out serum replacement (KSR), 1 × GlutaMax, 1 × MEM non-essential amino acids, 0.5 × penicillin and streptomycin, 1 × β-mercaptoethanol, and 1000 U/ml mouse LIF in knock-out DMEM (KO-DMEM), with serum-containing ESC medium containing 20% ES-FBS, 1 × GlutaMax, 1 × MEM non-essential amino acids, 0.5 × penicillin and streptomycin, 1 × β-mercaptoethanol, and 1000 U/ml mouse LIF in DMEM (all from Invitrogen, Carlsbad, CA).

2.3. Generation of human iPSCs

Human umbilical cord-derived mesenchymal stem cells (HUC-MSCs) were purchased from ATCC (Manassas, VA) and cultured in low serum mesenchymal stem cell growth kit (ATCC). Human neonatal foreskin keratinocytes (HNFKs) were purchased from Invitrogen and cultured in low calcium and serum-free Epilife medium (Invitrogen). For reprogramming, P4–P5 HUC-MSCs and P3 HNFKs were seeded the day before viral transduction, at 5×10^5 and 1×10^6 cells per 6-well plate, respectively. The next day (day 0), retrovirus-containing supernatant were added to the cells with 10 μg/ml polybrene and spinfected for 45 min at 650 g. The second spinfection was performed on the following day. Infected cells were passaged onto CF-1 feeders on day 3 in the presence of 10 μM Y-27632 (Merck Millipore, Billerica, MA) ROCK inhibitor. Culture medium was switched to a 1:1 mix of cell type specific medium and complete human ES medium on day 5. Starting from day 7, the medium was changed to complete human ES medium, which consists of 20% KSR, 0.5 × GlutaMax, 1 × non-essential amino acids, 0.5 × penicillin and streptomycin, 1 × β-mercaptoethanol and 4 ng/ml human bFGF in DMEM/F12. KO-DMEM was used in place of DMEM/F12 for HNFK reprogramming. Human iPSC colony formation was scored on day 10, 14 and 21 with NorthernLights NL557-conjugated TRA-1-60 live staining antibody (R&D systems, Minneapolis, MN). On day 21, alkaline phosphatase (AP) staining was performed using a Vector Red Alkaline Phosphatase Substrate Kit I (Vector Laboratories, Burlingame, CA). P1–P2 HUC-MSC derived iPSCs were cultured on mitomycin C-treated CF-1 feeder cells in complete human ES medium. For P2–P3, the human iPSCs were mechanically passaged onto matrigel-coated plates in mTeSR1 (Stemcell Technologies, Cambridge, MA) medium. The human iPSC lines from then on were expanded in mTeSR1 medium with 1 mg/ml dispase (Invitrogen) for passaging.

2.4. Embryoid body (EB) differentiation

Established human iPSCs at P6 and P7 in mTeSR1 medium were used for EB formation. iPSC colonies grew to 70–80% confluence were treated with 1 mg/ml dispase, washed three times with DMEM/F12 and plated for EB formation on low adhesive plastic petri dishes in DMEM/F12 medium with 20%KSR, 0.5 × GlutaMax, and 1 × MEM non-essential amino acids. EBs for qPCR were harvested for RNA isolation on day 5 in suspension culture. EBs for immunostaining were transferred on day 4 from suspension culture to 0.1% gelatin-coated plate for attachment and allowed to differentiate for one more week before being fixed for immunostaining as described below.

2.5. Immunostaining

Human EBs differentiated on 0.1% Gelatin in 12 well plates were fixed in 4% paraformaldehyde with 1% sucrose in PBS for 15 min at room temperature. The cell membranes were then permeabilized with 0.5% TX-100 in PBS-T, blocked in donkey serum and incubated with the antibodies provided in three germ layer 3-color immunocytochemistry kit (R&D system) for 2 h at 37 °C. The antibodies included the NL-