Biochemical and Biophysical Research Communications 443 (2014) 1206–1210

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Critical lysine residues of Klf4 required for protein stabilization and degradation

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ARTICLE INFO

Article history:
Received 19 December 2013
Available online 2 January 2014

Keywords:
Oncogene
Proteasomal degradation
Tumor suppressor
Ubiquitin
Ubiquitination

ABSTRACT

The transcription factor, Krüppel-like factor 4 (Klf4) plays a crucial role in generating induced pluripotent stem cells (iPSCs). As the ubiquitination and degradation of the Klf4 protein have been suggested to play an important role in its function, the identification of specific lysine sites that are responsible for protein degradation is of prime interest to improve protein stability and function. However, the molecular mechanism regulating proteasomal degradation of the Klf4 is poorly understood. In this study, both the analysis of Klf4 ubiquitination sites using several Klf4 deletion fragments and bioinformatics predictions showed that the lysine sites which are signaling for Klf4 protein degradation lie in its N-terminal domain (aa 1–296). The results also showed that Lys32, 52, 232, and 252 of Klf4 are responsible for the proteolysis of the Klf4 protein. These results suggest that Klf4 undergoes proteasomal degradation and that these lysine residues are critical for Klf4 ubiquitination.

1. Introduction

The ubiquitin proteasome pathway of intracellular proteolysis controls the fate of proteins and the regulation of cellular homeostasis. Ubiquitin conjugation to target proteins at lysine residues is tightly regulated by three sequential ubiquitin-activating (E1), conjugating (E2), and ligating (E3) enzyme reactions through an ATP-dependent manner [1,2]. Ubiquitin can establish chains through the addition of free ubiquitins on its lysine (K) residues by E2 and E3 enzymes [3]. The products of ubiquitin-catalyzed proteins are mono-ubiquitination, multiple mono-ubiquitination, or poly-ubiquitination chain conformations [3,4]. Ubiquitin consists of 76 amino acids and has seven lysine residues; K6, K11, K27, K29, K33, K48, and K63 [3]. K48-linked polyubiquitin chain-targeted proteins undergo the 26S proteasomal degradation and regulate the half-life of proteins, and K63-linked polyubiquitin chains mediate intercellular signaling transduction [3,5,6]. Ubiquitin chains can attach to target proteins as various forms; a single mono-ubiquitin, a multiple mono-ubiquitin, a single ubiquitin chain, a multiple ubiquitin chain, and a mixed mono- or poly-ubiquitin chain [5]. Proteins with ubiquitin-binding domains can recognize and interact with those ubiquitins [7].

Induced pluripotent stem cells (iPSCs) are dedifferentiated from somatic cells through reverse differentiation and reprogramming, and they can exhibit pluripotency. It has been well established that differentiated somatic cells can be induced into iPSCs following the expression of Oct4, Sox2, Klf4 and c-Myc (OSKM) [8,9]. Further, several technical methods and research tools have been applied to establish more efficient iPSCs [10]. Recent studies showed that recombinant proteins of OSKM can be used for making protein-induced human iPSCs (p-hiPSCs) and that these p-hiPCSs showed long-term self-renewal and pluripotency [11,12]. These results raise the possibility of more stable recombinant OSKM, increasing pluripotency. Despite the use of recombinant proteins for iPSCs, the analysis of proteasomal degradation and ubiquitination patterns of OSKM are not yet fully understood. Several studies have provided a line of evidence for OSKM ubiquitination. It has been demonstrated that Oct-4 undergoes sumoylation and that Wwp2 promotes Oct4 degradation by ubiquitination [13–15]. Moreover, a recent study showed that itch, an Oct4 specific E3 ligase, regulates Oct4 ubiquitination and degradation [16]. Additionally, Klf4 underwent the proteasomal degradation by serum stimulation [17]. We recently revealed that the transcription factor Nanog required for pluripotency undergoes proteasomal degradation through its PEST motif (rich in proline, glutamic acid, serine, and threonine) [18]. In this study, we identified critical lysine sites on Klf4 involved in its ubiquitination. We also demonstrated that the stability of a lysine mutated Klf4 protein was increased, compared with wild-type Klf4. Our findings might help to support advances in generating iPSCs via the ubiquitin-proteasome pathway.

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0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.12.121

2. Materials and methods

2.1. Cell culture, transfection, reagents, and antibodies

Human embryonic kidney (HEK) 293T cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) and 1% penicillin–streptomycin (Gibco, Grand Island, NY, USA). Polyethylenimine (PEI) (Polyscience, Warrington, PA, USA) was used for the cell transfection, and DNA ratio to PEI of N/P = 20 was treated. After treatment of the cells with the DNA/PEI, cells were incubated at 37 °C overnight. After incubation, the cells were washed once with PBS and grown in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin. A total of 100 μg/mL of cycloheximide (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in distilled water and added to the cells 24 h after transfection. Anti-Myc (9E10) and anti-HA antibodies (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used for immune blotting.

2.2. Generation of expression plasmids

The full-length cDNA for human Klf4 was cloned into a Myc-tagged pcDNA3.1 expression vector (Invitrogen, Carlsbad, CA, USA). Each deletion fragment of Klf4 ($Klf4_{1-420}$, $Klf4_{1-296}$, $Klf4_{1-204}$, $Klf4_{190-479}$, and $Klf4_{263-479}$) was subcloned into a Myc-tagged pcDNA3.1 expression vector. Multiple lysine mutants of Klf4 were generated using site-directed mutagenesis with Pfu polymerase (Enzynomics, Daejeon, Korea) according to the manufacturer's protocol [18], and specific primers were targeted by polymerase chain reaction (PCR). The HA-tagged ubiquitin construct was previously described [18].

2.3. Immunoblotting and in vivo ubiquitination assay

For immunoblotting, the cells were washed twice with ice-cold PBS, and then lysed in Triton X-100 lysis buffer (50 mM Tris–HCl [pH 8.0], 150 mM NaCl, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF) and a protease inhibitor cocktail (PIC) tablet (Roche, Mannheim, Germany) in PBS). Protein expression analysis was performed with anti-Myc (9E10, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-HA (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-ubiquitin (Cell signaling Technology, Beverly, MA, USA), and anti-β-actin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). For the *in vivo* ubiquitination assay, the cells were resuspended in a lysis buffer (50 mM Tris–HCl [pH 7.5],

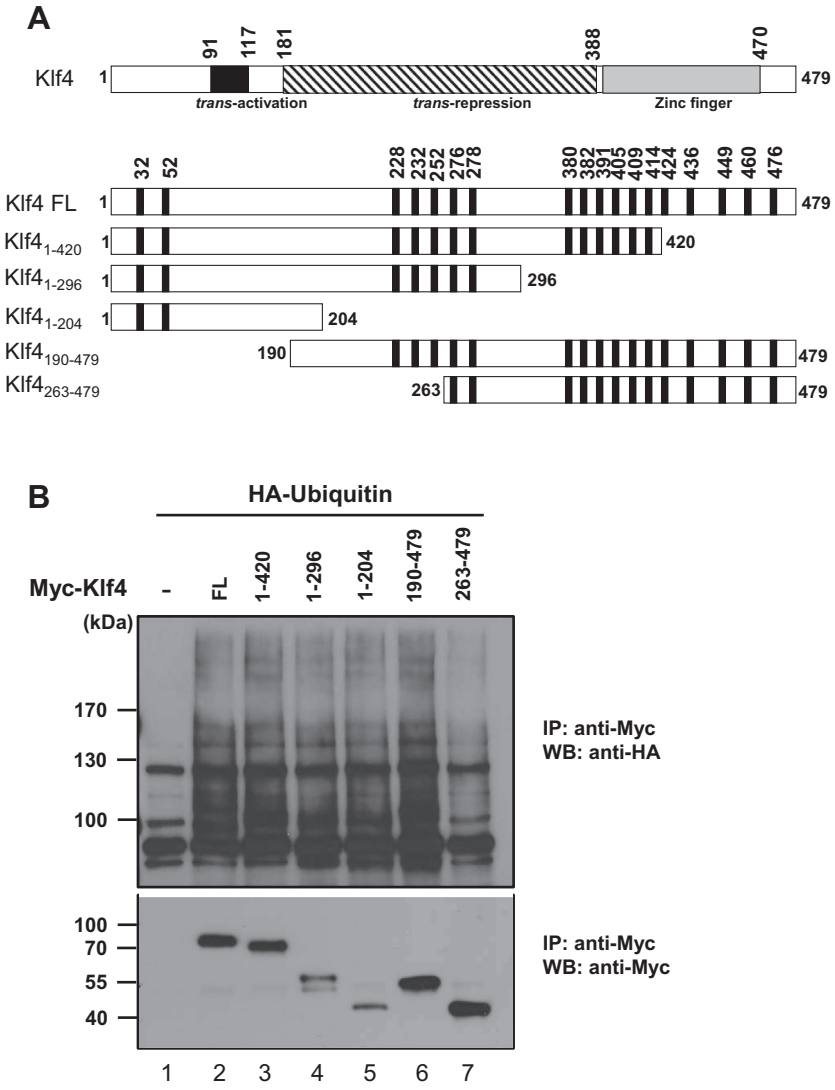

Fig. 1. Klf4 contains 18 lysine sites and its partial peptides showed ubiquitination. (A) Schematic representation of Klf4 lysine residues and the five deletion constructs. (B) HEK 293 cell extracts that were co-transfected with five Myc-tagged Klf4 deletion constructs and HA-tagged ubiquitin were immunoprecipitated with an anti-Myc antibody, and pull-downed precipitates were analyzed by immunoblotting with an anti-HA antibody.

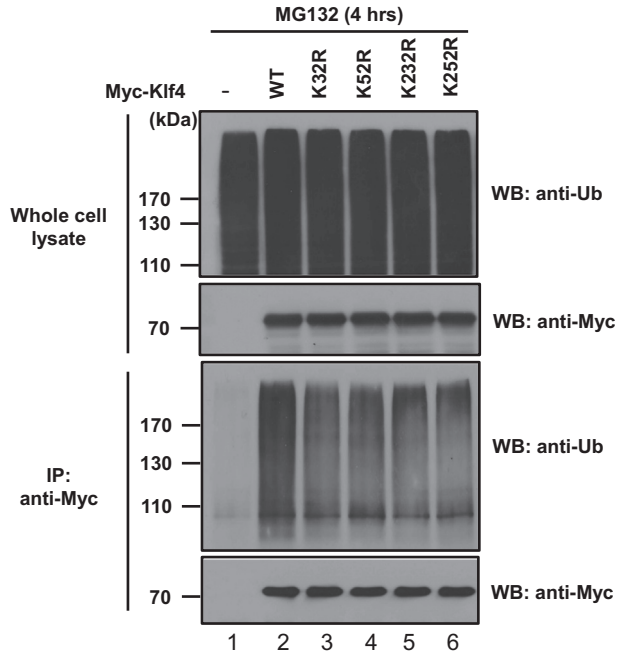

Fig. 2. Lys32, Lys232 and Lys252 were critical sites of ubiquitination of Klf4. HEK 293T cells were transfected with Myc-tagged Klf4-wild-type, and Myc-tagged-K32R, -K52R, -K232R and -K252R. These transfected cells were treated with MG132 4 h before harvest. Cell lysates were immunoprecipitated with an anti-Myc antibody, and immunoprecipitates were detected by immunoblotting with anti-ubiquitin and anti-Myc antibodies.

150 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 1 mM PMSF, 0.1% SDS and a PIC) and incubated on ice for 30 min. The supernatants from the cell pellets were incubated with antibodies overnight at 4 °C, and protein A/G Sepharose (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was added to the supernatants and incubated at 4 °C for 1 h. The ubiquitinated proteins were separated by 8% SDS–PAGE gel and then detected by immunoblotting with appropriate antibodies.

3. Results

3.1. Mapping of ubiquitination sites on Klf4

Previously, a structural analysis of Klf4 showed that Klf4 has zinc finger, *trans*-activation, and *trans*-repression functional domains (Fig. 1A) [19]. Moreover, a recent study reveled that Klf4 undergoes ubiquitination and proteolysis by the 26S proteasome [20]. The E3 ligase plays an essential role in forming an isopeptide bond between the C-terminus of ubiquitin and lysine residues on its substrates [21]. To identify the ubiquitination site on Klf4, we analyzed lysine sites in human Klf4, which has 18 lysines (Fig. 1A). Interestingly, most of the lysine residues were accumulated in *trans*-repression and zinc finger domains at the C-terminal region of Klf4 (Fig. 1A). We referred to two bioinformatics databases, UbPred (http://www.ubpred.org) and NetChop (http://www.cbs.dtu.dk/services/NetChop), to predict the putative lysine sites responsible for polyubiquitination of Klf4 [22]. Following the comparison of the data in the two databases, we selected overlapping potential lysine sites for Klf4 ubiquitination. Although most of the lysine sites were located in the C-terminal region of Klf4, high-scored putative ubiquitination sites resided towards the proximal and distal regions in the N-terminus of Klf4. To confirm this finding, we generated five fragments of Klf4, 1–420, 1–296, 1–204, 190–479, and 263–479, and we tested the level of ubiquitination in each fragment (Fig. 1A and B). The ubiquitination analysis with the five Klf4 fragments showed that ubiquitin was accumulated in the $Klf4_{190-479}$ region (Fig. 1B, lane 6). We observed the weakest ubiquitin signal in the $Klf4_{263-479}$ fragment, indicating that Klf4 ubiquitination mainly arises in lysine sites that are located between amino acid 190–263. In addition, we observed that the ubiquitination level of the $Klf4_{1-204}$ and $Klf4_{1-296}$ mutants was less than that of the full-length Klf4 but that their expressions were

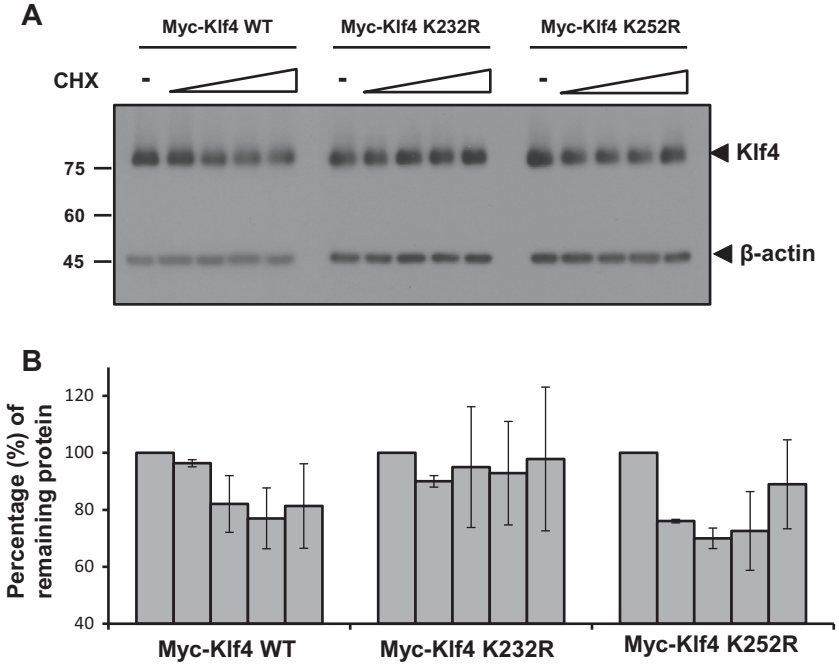

Fig. 3. The Klf4 mutations K232 and K252 inhibited protein degradation. (A) HEK 293T cells were transfected with wild-type Myc-tagged Klf4, and Myc-tagged-K232R and -K252R mutants, and treated with cycloheximide (CHX) in a gradient manner (50, 100, 200, 400 μg/mL) for 6 h. The cell extracts were separated by SDS–PAGE and immunoblotted with anti-Myc and anti-β-actin antibodies. (B) Data are mean ± standard errors of three independent experiments.

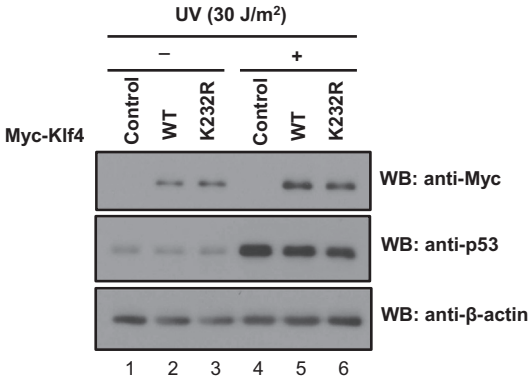

Fig. 4. The Lys232 mutant of Klf4 suppressed p53 expression. Control or UV (30 J/m^2) treated MCF7 cells that expressed wild-type Myc-tagged Klf4 and a Myc-tagged-K232R mutant were analyzed by Western blotting with anti-Myc, anti-p53, and anti-β-actin antibodies.

significantly decreased. Together, the results indicate that Klf4 undergoes 26S proteasomal degradation and that lysines in the Klf4$_{190\text{-}263}$ region are essential for protein degradation.

3.2. Identification of critical lysine residues of Klf4 for ubiquitination

Ubiquitin usually makes an isopeptide bond between its C-terminus and lysine sites on target proteins by E3 ubiquitin ligases [23]. In this study, we found that the downstream region of Klf4$_{1-296}$ is crucial for ubiquitination, as shown in Fig. 1B. Thus, it is expected that K232 and K252 are critical sites for Klf4 ubiquitination. To define the critical lysine residue of Klf4, we generated four mutants, substituting lysine (K) with arginine (R) on Klf4 through site-directed mutagenesis (SDM). To identify the essential lysine site on Klf4 for ubiquitination, we determined the ubiquitination levels of the lysine mutants of Klf4. The ubiquitination of wild-type Klf4 and all lysine mutated Klf4 was increased by MG132, a proteasome inhibitor, and we observed some reduction in the ubiquitination pattern of the four Klf4 mutants compared with wild-type Klf4 (Fig. 2, lanes 3–6). In particular, K232R and K252R mutants of Klf4 strongly inhibited ubiquitination (Fig. 2, lane 3). These data indicate that Lys232 and 252 are critical lysine residues for Klf4 ubiquitination.

3.3. Stability of Klf4 mutants in proteasomal degradation

The ubiquitination analysis of Klf4 revealed that the Klf190–296 domain is a crucial region for Klf4 ubiquitination and that Lys232 and Lys252 residues specifically regulate Klf4 ubiquitination. Based on this observation, we tested whether Lys mutants of Klf4 show resistance to proteasomal degradation. We treated cells overexpressing each Klf4 and Lys232 and Lys252 mutants of Klf4 with cycloheximide (CHX), a protein synthesis inhibitor (Fig. 3). The results indicated that the half-life of the Lys232 mutant of Klf4 was extended compared with that of wild-type Klf4. These data suggest that the Lys232 residue of Klf4 is critical for the ubiquitination of the Klf4 protein. The tumor suppressor p53 is known as a down-regulator of Klf4, and overexpression of Klf4 repressed p53 expression [24]. We next examined whether the lysine mutated Klf4 suppressed p53 expression. We overexpressed wild-type Klf4 and the Lys232 mutant of Klf4 in wild-type p53 expression cell line (MCF7) (Fig. 4). As expected, Lys232 mutated Klf4 exhibited decreased p53 expression (Fig. 4, lane 3). Moreover, p53 expression was suppressed in the Klf4 mutant exposed to ultraviolet irradiation (Fig. 4, lane 6).

4. Discussion

Four transcription factors of OSKM are known to induce iPSCs from murine somatic cells [8]. Reprogramming factors, cell delivery systems, cell types, cell culture conditions and factor expression to generate iPSCs from differentiated cells are regarded as important points in improving the generation of iPSCs [25]. Recently, several studies used recombinant-reprogramming proteins called protein-induced pluripotent stem cells (piPSCs) [11,12]. These studies showed the significant potential of *in vivo* or *in vitro* reprogramming proteins in improving the generation of iPSCs. Although many researches have focused on generating iPSCs with various protocols [10], the study of the stability and ubiquitination patterns of the four factors (OSKM transcription factors) are poorly understood. Interestingly, research on c-Myc degradation has revealed interesting findings. For example, c-Myc was shown to contain two conserved motifs, myc homology box I (MBI, amino acids 45–65) and myc homology box II (MBII, amino acids 128–144), both of which were targeted during proteasomal degradation [26]. Another study showed that the N-terminal deletion (amino acids 1–147) of c-Myc inhibited ubiquitination and increased protein stability [27]. Moreover, missense amino acid mutations accumulated in the c-Myc MBI domain in primary Burkitt's and AIDS-associated lymphomas [28]. Recently, we demonstrated that the PEST motif is an important site for Nanog ubiquitination and degradation and that deletion of PEST increased Nanog stability [18]. We assumed that increasing the stability of reprogramming proteins by inhibition of their ubiquitination would prevent protein turnover and produce materials more suited to the generation of iPSCs.

Approximately, 25 human Klf family genes are located throughout the human genome [29]. Several Klfs are associated with the regulation of cellular proliferation. For example, Klf4 and Klf5 have been introduced as yin and yang regulators with tumor suppressor and oncogene in regulation for cancer cell proliferation [30]. Klf6 has been investigated as a tumor suppressor gene, and a mutation of Klf5 was found in prostate cancer [31,32]. Interestingly, several studies reported that Klf4 has oncogenic function [24,29,33]. These contrary results for Klf4 functions suggested one possibility that it acts as a switching-protein between tumor suppression and oncogenic function via its expression pattern [29]. Recently, a study revealed that the mutant for sumoylation site of Klf4 suppressed p21 protein expression, and it has the same effect as wild-type Klf4 for iPSC induction [34]. In the present study, we revealed that the region of Klf4 190–263 is critical for ubiquitination and degradation by the 26S proteasome through a proteomic analysis approach. Moreover, we found that Lys232 on Klf4 is a main ubiquitination amino acid residue of Klf4 and that a mutation of Lys232 on Klf4 enhances protein stability in the cells. In addition, we found that a Lys232 mutation of Klf4 represses the expression of p53. Collectively, these results suggest that the post-translational modification might be useful for generating a stable protein to induce iPSCs. In addition, our results may help to understand the role of different Klf4 expression patterns and their functions in cellular regulation.

Acknowledgment

This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korea government (MEST) (NRF-2011-0015312).

References

[1] A. Hershko, A. Ciechanover, The ubiquitin system, Annu. Rev. Biochem. 67 (1998) 425–479.

[2] C.M. Pickart, M.J. Eddins, Ubiquitin: structures, functions, mechanisms, Biochim. Biophys. Acta 1695 (2004) 55–72.
[3] F. Ikeda, I. Dikic, Atypical ubiquitin chains: new molecular signals. 'Protein Modifications: Beyond the Usual Suspects' review series, EMBO Rep. 9 (2008) 536–542.
[4] C.M. Pickart, D. Fushman, Polyubiquitin chains: polymeric protein signals, Curr. Opin. Chem. Biol. 8 (2004) 610–616.
[5] I. Dikic, S. Wakatsuki, K.J. Walters, Ubiquitin-binding domains – from structures to functions, Nat. Rev. Mol. Cell Biol. 10 (2009) 659–671.
[6] I.E. Wertz, V.M. Dixit, Regulation of death receptor signaling by the ubiquitin system, Cell Death Differ. 17 (2010) 14–24.
[7] J.W. Harper, B.A. Schulman, Structural complexity in ubiquitin recognition, Cell 124 (2006) 1133–1136.
[8] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126 (2006) 663–676.
[9] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, S. Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (2007) 861–872.
[10] F. Gonzalez, S. Boue, J.C. Izpisua Belmonte, Methods for making induced pluripotent stem cells: reprogramming a la carte, Nat. Rev. Genet. 12 (2011) 231–242.
[11] H. Zhou, S. Wu, J.Y. Joo, S. Zhu, D.W. Han, T. Lin, S. Trauger, G. Bien, S. Yao, Y. Zhu, G. Siuzdak, H.R. Scholer, L. Duan, S. Ding, Generation of induced pluripotent stem cells using recombinant proteins, Cell Stem Cell 4 (2009) 381–384.
[12] D. Kim, C.H. Kim, J.I. Moon, Y.G. Chung, M.Y. Chang, B.S. Han, S. Ko, E. Yang, K.Y. Cha, R. Lanza, K.S. Kim, Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins, Cell Stem Cell 4 (2009) 472–476.
[13] F. Wei, H.R. Scholer, M.L. Atchison, Sumoylation of Oct4 enhances its stability, DNA binding, and transactivation, J. Biol. Chem. 282 (2007) 21551–21560.
[14] H. Xu, W. Wang, C. Li, H. Yu, A. Yang, B. Wang, Y. Jin, WWP2 promotes degradation of transcription factor OCT4 in human embryonic stem cells, Cell Res. 19 (2009) 561–573.
[15] B. Liao, Y. Jin, Wwp2 mediates Oct4 ubiquitination and its own auto-ubiquitination in a dosage-dependent manner, Cell Res. 20 (2010) 332–344.
[16] B. Liao, X. Zhong, H. Xu, F. Xiao, Z. Fang, J. Gu, Y. Chen, Y. Zhao, Y. Jin, Itch, an e3 ligase of oct4, is required for embryonic stem cell self-renewal and pluripotency induction, J. Cell Physiol. 228 (2013). in press.
[17] Z.Y. Chen, X. Wang, Y. Zhou, G. Offner, C.C. Tseng, Destabilization of Kruppel-like factor 4 protein in response to serum stimulation involves the ubiquitin-proteasome pathway, Cancer Res. 65 (2005) 10394–10400.
[18] S. Ramakrishna, B. Suresh, K.H. Lim, B.H. Cha, S.H. Lee, K.S. Kim, K.H. Baek, PEST motif sequence regulating human NANOG for proteasomal degradation, Stem Cells Dev. 20 (2011) 1511–1519.
[19] Z. Cao, X. Sun, B. Icli, A.K. Wara, M.W. Feinberg, Role of Kruppel-like factors in leukocyte development, function, and disease, Blood 116 (2010) 4404–4414.
[20] D. Hu, Z. Zhou, N.E. Davidson, Y. Huang, Y. Wan, Novel insight into KLF4 proteolytic regulation in estrogen receptor signaling and breast carcinogenesis, J. Biol. Chem. 287 (2012) 13584–13597.
[21] C.M. Pickart, Mechanisms underlying ubiquitination, Annu. Rev. Biochem. 70 (2001) 503–533.
[22] P. Radivojac, V. Vacic, C. Haynes, R.R. Cocklin, A. Mohan, J.W. Heyen, M.G. Goebl, L.M. Iakoucheva, Identification, analysis, and prediction of protein ubiquitination sites, Proteins 78 (2010) 365–380.
[23] D. Komander, M. Rape, The ubiquitin code, Annu. Rev. Biochem. 81 (2012) 203–229.
[24] B.D. Rowland, R. Bernards, D.S. Peeper, The KLF4 tumour suppressor is a transcriptional repressor of p53 that acts as a context-dependent oncogene, Nat. Cell Biol. 7 (2005) 1074–1082.
[25] N. Maherali, K. Hochedlinger, Guidelines and techniques for the generation of induced pluripotent stem cells, Cell Stem Cell 3 (2008) 595–605.
[26] E.M. Flinn, C.M. Busch, A.P. Wright, Myc boxes, which are conserved in myc family proteins, are signals for protein degradation via the proteasome, Mol. Cell Biol. 18 (1998) 5961–5969.
[27] S.E. Salghetti, S.Y. Kim, W.P. Tansey, Destruction of Myc by ubiquitin-mediated proteolysis: cancer-associated and transforming mutations stabilize Myc, EMBO J. 18 (1999) 717–726.
[28] F. Bahram, N. von der Lehr, C. Cetinkaya, L.G. Larsson, C-Myc hot spot mutations in lymphomas result in inefficient ubiquitination and decreased proteasome-mediated turnover, Blood 95 (2000) 2104–2110.
[29] B.D. Rowland, D.S. Peeper, KLF4, p21 and context-dependent opposing forces in cancer, Nat. Rev. Cancer 6 (2006) 11–23.
[30] A.M. Ghaleb, M.O. Nandan, S. Chanchevalap, W.B. Dalton, I.M. Hisamuddin, V.W. Yang, Kruppel-like factors 4 and 5: the yin and yang regulators of cellular proliferation, Cell Res. 15 (2005) 92–96.
[31] G. Narla, K.E. Heath, H.L. Reeves, D. Li, L.E. Giono, A.C. Kimmelman, M.J. Glucksman, J. Narla, F.J. Eng, A.M. Chan, A.C. Ferrari, J.A. Martignetti, S.L. Friedman, KLF6, a candidate tumor suppressor gene mutated in prostate cancer, Science 294 (2001) 2563–2566.
[32] G. Narla, S.L. Friedman, J.A. Martignetti, Kruppel cripples prostate cancer: KLF6 progress and prospects, Am. J. Pathol. 162 (2003) 1047–1052.
[33] C. Le Magnen, L. Bubendorf, C. Ruiz, I. Zlobec, A. Bachmann, M. Heberer, G.C. Spagnoli, S. Wyler, C. Mengus, Klf4 transcription factor is expressed in the cytoplasm of prostate cancer cells, Eur. J. Cancer. 49 (2013) 955–963.
[34] S. Tahmasebi, M. Ghorbani, P. Savage, K. Yan, G. Gocevski, L. Xiao, L. You, X.J. Yang, Sumoylation of Kruppel-like factor 4 inhibits pluripotency induction but promotes adipocyte differentiation, J. Biol. Chem. 288 (2013) 12791–12804.