Genomics Proteomics Bioinformatics 11 (2013) 294–298

Genomics Proteomics Bioinformatics

www.elsevier.com/locate/gpb
www.sciencedirect.com

REVIEW

Clinical Therapy Using iPSCs: Hopes and Challenges

Xiao Lu, Tongbiao Zhao *

State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

Received 9 August 2013; revised 4 September 2013; accepted 4 September 2013
Available online 21 September 2013

KEYWORDS

iPSC;
Regenerative medicine;
Disease modeling;
Immunogenicity;
Tumorigenicity

Abstract Induced pluripotent stem cells (iPSCs) are generated by ectopic expression of defined transcription factors in somatic cells. They can undergo unlimited self-renewal and maintain the embryonic stem cells (ESCs)-like ability to differentiate into all three germ layers. iPSCs can potentially provide unlimited autologous cells for therapy and therefore hold great promise for regenerative medicine. Here we reviewed the recent advances in iPSC studies on disease modeling and clinical treatment as well as challenges correlated with clinical development of iPSCs, like tumorigenicity, immunogenicity and genomic instability.

Introduction

In 2006, using Fbx15βgeo as a reporter system, Yamanaka's group screened a panel of genes specifically expressed in embryonic stem cells (ESCs) and determined that four transcription factors— Oct4, Sox2, Klf4 and c-Myc— are sufficient to reprogram mouse fibroblasts into pluripotent stem cells, which we called induced pluripotent stem cells (iPSCs) [1]. The same cocktail can also reprogram human differentiated fibroblast into iPSCs [2–5]. In 2007, Thomson's group identified another combination — Oct4, Sox2, Lin28 and Nanog — that can induce human somatic cells to become pluripotent [6]. Like ESCs, iPSCs can undergo unlimited self-renewal and maintain the ability to differentiate into all three germ layers. iPSCs can not only contribute to chimerism and germ line transmission in mice, but also can develop into full-term iPSC mice by tetraploid complementation [1–3,7–10], indicating the totipotency of iPSCs.

Somatic cell conversion to the iPSC state is accompanied by epigenetic remodeling, including resetting of the chromatin structure and methylation states of DNA and histone. The process of cell fate switching that culminates in the iPSC phenotype makes this type of cell an ideal model for studying basic biological phenomena such as development and differentiation. The low efficiency of reprogramming and long period of time required for reprogramming to occur complicate efforts to study the mechanism of reprogramming, which has been widely discussed and reviewed [11–14]. In this review, instead of discussing the reprogramming mechanisms, we focus on the promises and challenges of using iPSCs therapeutically.

Disease modeling

Theoretically, patient-specific iPSCs can be obtained and differentiated into different cell types with the same genetic background as the donor patient, providing the opportunity to study pathogenesis *in vitro*, so-called "modeling disease in a dish". Indeed, iPSCs have already been derived from patients with a large variety of diseases [12]. It is very challenging to study the pathogenesis of human neurological disease, due to

* Corresponding author.
E-mail: tbzhao@ioz.ac.cn (Zhao T).

Peer review under responsibility of Beijing Institute of Genomics, Chinese Academy of Sciences and Genetics Society of China.

ELSEVIER Production and hosting by Elsevier

1672-0229/$ - see front matter © 2013 Beijing Institute of Genomics, Chinese Academy of Sciences and Genetics Society of China. Production and hosting by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gpb.2013.09.002

the complexity of the neuronal system and the difficulty of culturing neurons *in vitro*. iPSCs are a practical means of studying the development and function of human neurons. Spinal muscular atrophy was the first neurological disease targeted in a human iPSC-based study of pathogenesis [15]. The patient-derived iPSCs generated in the study gave rise to motor neurons with the same genotype that is associated with selective deficits, providing the proof of concept that iPSCs can be used to model human disease [15]. In another study, iPSCs derived from Rett syndrome patients were not only able to recapitulate the hallmark defects associated with the disease but were also used to test the effects of drugs in rescuing synaptic defects [16].

Recent studies in which iPSCs have been derived from patients with Huntington-Gilford progeria syndrome (HGPS) have shown that the smooth muscles derived from the patient iPSCs recapitulated the premature senescence *in vitro*, suggesting great promise for elucidating the molecular mechanisms underlying the HGPS disease by using iPSCs [17,18]. Interestingly, Liu et al. demonstrated the contribution of the LRRK2 G2019S mutation to Parkinson's disease (PD), and for the first time showed that nuclear-envelope defects might be involved in PD pathology, opening new avenues for PD diagnoses and treatment [19]. iPSCs have also been used to model cardiac disease. iPSCs derived from patients with long-QT syndrome were induced to differentiate into functional cardiac myocytes that recapitulated the electrophysiological defects characteristic of the disorder [20].

Using iPSC-derived patient-specific cells to model an adult-onset disease remains challenging, owing to the difficulties involved in differentiating the iPSCs into an adult organ and the complexity of pathogenesis associated with development. Kim et al. provided the first evidence that induction of adult-like metabolism has a crucial role in establishing the adult-onset disease arrhythmogenic right ventricular dysplasia (ARVD) using patient-specific iPSCs [21].

Those preliminary studies inspired more-extensive disease modeling studies using iPSCs. To date, dozens of disorders affecting neurons, blood, liver, heart, pancreas, lung as well immunological disorders and cancer were studied by using iPSCs [12,22,23]. Lack of appropriate model systems is a major block to the study of human hepatitis C virus (HCV) infection in humans. Interestingly, it was recently reported that hepatocyte-like cells derived from iPSCs can support the entire life cycle of HCV in humans, validating the feasibility of using iPSC as a model system to study human HCV infection [22,24]. In support of this idea, another recent study showed that hepatic cells derived from pigtail macaque can also support HCV infection [25].

Despite plenty of disease modeling using cells differentiated from iPSCs, generation of complex three-dimensional organs and tissues for regenerative medicine is still a major challenge. Two inspiring studies showed that three-dimensional intestine and liver can be derived from iPSCs [26,27], providing proof-of-concept that iPSCs can be used to generate functional organs *in vitro* for regenerative medicine.

iPSCs for therapy

The first proof-of-principle experiment involving the use of iPSCs to cure disease was performed by the Jaenisch lab, using a humanized sickle cell anemia mouse model [28]. Hanna et al. first derived the mouse iPSCs, corrected the sickle hemoglobin allele by gene-specific targeting, differentiated the iPSCs into hematopoietic progenitors, and then transplanted these corrected progenitors into the mice. This strategy successfully rescued the phenotype of the blood cells [28]. By transplanting human iPSC-derived multipotent cardiovascular progenitor cells into mouse, Lu et al demonstrated that the transplanted cardiovascular progenitor cells can migrate, proliferate and differentiate *in situ* into cardiomyocytes, smooth muscle cells and endothelial cells to reconstruct the damaged heart [29].

The clinical development of human iPSCs for therapy is still in its preliminary stage. There are two encouraging clinical trials using human ESC (hESC)-derived cells for therapy in the USA that have been approved by the FDA. Geron performed the first FDA-approved clinical trials using hESC-derived cells to treat spinal cord injury (www.clinicaltrials.gov). Another, more-encouraging trial was performed by Advanced Cell Technology (ACT), using hESC-derived retinal pigment epithelial (RPE) cells to treat macular degeneration (MD). Although no significant vision improvement has been observed four months after transplantation, structural evidence confirms that cells have attached and continued to persist in the treated patients. Most importantly, no hyperproliferation, abnormal growth, or immune-mediated transplant rejection was observed in these transplanted patients, and no patients lost their vision during the first four months [25,30]. Subsequent clinical observations are expected. Meanwhile, a preliminary clinical trial for transplantation of iPSC-derived RPE cells was performed by Masayo Takahashi in Japan, as reported in the ISSCR 2012 Annual Meeting. Publication of those clinical data is eagerly expected.

Tumorigenicity

The boosting of patient-specific iPSC derivation and iPSC-based disease modeling underscores the great potential use of this technology in regenerative medicine. However, to translate the iPSC technology to therapy quickly, extensive preclinical experiments are required to evaluate the safety and effectiveness of this new type of therapy.

The first generation of iPSCs were obtained by overexpressing the defined transcription factors, using retrovirus or lentivirus [1,2,5,6]. Integration of the viral genome into that of the host poses a serious cancer risk [7,31]. iPSCs were subsequently generated without viral integration by using piggyBac transposition [32,33]. Soon after, adenovirus or plasmid or episomal vector transfection was successfully used to reprogram fibroblast into iPSCs, both in mouse and human [34–40]. Moreover, two recent studies showed that both mouse and human iPSCs can be obtained by directly delivering reprogramming factor proteins into target cells without any DNA manipulation [41,42]. All of the aforementioned methods used for reprogramming can be applied to generate iPSCs without exogenous DNA integration into the host genome. Remarkably, tumors were not observed in mice derived from integration-free iPSCs up to 20 weeks of age [35]. Recently, mouse iPSCs were generated by adding only seven small molecule compounds into the cell culture, suggesting the possibility of generating human iPSCs for clinical application without tedious genetic manipulations [43]. This study indicates that cell fate decisions can be

dictated by manipulating intrinsic signal pathways, and represents an innovative breakthrough in the understanding of reprogramming mechanisms.

The cancer risks raised by virus or reprogramming factor integration into the genome could be averted by the use of integration-free reprogramming technologies. However, whether reprogramming itself can lead to tumorigenesis is still unknown.

The iPSC reprogramming factors have clearly demonstrated oncogenicity. Oct4 was shown to dictate the oncogenic potential of ESCs in a dose–dependent way. Overexpression of Oct4 enhances the malignant potential of ESC-derived tumors, while inactivation decreases malignant potential [44–46]. Sox2 is a lineage-survival oncogene in lung and esophageal squamous cell carcinomas [47]. Klf4 functions as a tumor suppressor gene and oncogene in a context-dependent manner [48]. c-Myc is an oncogene as well [49]. Nanog expression has been detected in various tumors and is thought to be an oncogene [50,51]. Recent studies showed that Nanog can promote breast cancer tumorigenesis and metastasis [52]. The overexpression of oncogenic genes can potentially make cells grow out of control and cancerous. The oncogenicity of reprogramming factors can transform some of cells during reprogramming [53]. During reprogramming, some ES-like colonies failed to expand when the original "iPS" colonies were picked up, other ES-like colonies can be expanded but lack pluritpotency-defined partially reprogrammed iPSCs [53]. Whether the bulk transfection of oncogenic reprogramming factors into somatic cells can cause abnormality in iPSCs is still under investigation.

Immunogenicity

Although it is widely assumed that iPSC-derived autologous cells are immune privileged, the immunogenicity of cells differentiated from iPSCs is not extensively studied. Recently, we first showed that iPSC derivatives can elicit immune rejection response when transplanted to the syngeneic mice by using a teratoma model [40]. Although two following-up studies claimed either "negligible" or "lack of" immunogenicity of iPSC derivatives, they both support that some certain tissues but not all tissues differentiated from iPSCs are immunogenic. Abe group clearly showed that the cardiomyocytes differentiated from iPSCs can elicit immune rejection responses (please refer to Sup Fig. 13) [54]. Recent report by Guha et al. clearly showed the immunogenicity differences between ESC- and iPSC- derived endoderm cells [55].

It should be noted that (1) only certain but not all tissues derived from iPSCs can elicit immune rejection response; (2) the rejection intensity induced by ESC-derived allografts differs from that induced by iPSC-derived autografts, due to the fact that MHC-I molecules are expressed in all allogeneic ESC-derived cells and only certain syngeneic iPSC derivatives can express minor antigens; (3) if a specific autologous cell type derived from iPSCs is immunogenic, it is capable of eliciting serious minor antigen-induced rejection of the cells. In general, we can still take easier advantage of iPSCs for therapy than allogeneic ESC lines even when immunologic issues are considered.

Genomic instability

Many studies have identified chromosomal abnormalities in iPSCs, indicating that reprogramming itself can induce genetic instability. Recently, sub-karyotype abnormalities were defined in multiple iPSC lines by using Array Comparative Genomic Hybridization (aCGH) [56]. Comparative genomic hybridization analysis of iPSCs revealed the presence of genomic deletions and amplifications, suggesting oncogene-induced DNA replication stress during reprogramming [57]. High-resolution single nucleotide polymorphism (SNP) analysis revealed a higher frequency of subchromosomal copy number variations (CNVs) in human iPSCs compared to somatic cells [58]. Similarly, another study showed that early-passage human iPSCs harbor significantly more chromosomal CNVs than do intermediate human iPSCs, fibroblasts or human ESCs. Interestingly, in that study, iPSCs with CNVs were rapidly disappeared in the iPSC pool during expansion [59]. By using deep sequencing, a recent study detected somatic coding mutations in human iPSCs, suggesting that human iPSCs acquire not only epigenetic but also genetic modifications [60].

The tumor suppressor p53 functions as the guardian of the genome, as it is involved in DNA damage response, cell cycle arrest, senescence and apoptosis [61–63]. Recent studies clearly showed that p53 is a barrier to reprogramming [64–69]. Inhibition of p53 activity can enhance reprogramming efficiency. However, whether inactivation of p53 is required for reprogramming and whether p53 inactivation directly contributes to genomic instability in iPSCs are questions still under investigation.

Perspective

Although significant progress has been made in understanding tumorigenicity, immunogenicity and genomic instability in iPSCs, the relationship among these abnormalities and how to overcome the associated hurdles for clinical development of iPSCs are still undergoing study. Meanwhile, encouraging progress in the development of integration-free reprogramming approaches, disease modeling, and preclinical trials has significantly enhanced the prospects of advancing iPSC technology from bench to bedside.

Competing interests

The authors declared that no competing interests exist.

Acknowledgements

This work was supported by grants from the National Basic Research Program of China (Grant No. 2012CB966901), the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA01040108) and the National Natural Science Foundation of China (Grant No. 31271592) to TZ.

References

[1] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–76.

[2] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–72.

[3] Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, et al. *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature 2007;448:318–24.
[4] Lowry WE, Richter L, Yachechko R, Pyle AD, Tchieu J, Sridharan R, et al. Generation of human induced pluripotent stem cells from dermal fibroblasts. Proc Natl Acad Sci U S A 2008;105:2883–8.
[5] Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008;451:141–6.
[6] Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318:1917–20.
[7] Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007;448:313–7.
[8] Boland MJ, Hazen JL, Nazor KL, Rodriguez AR, Gifford W, Martin G, et al. Adult mice generated from induced pluripotent stem cells. Nature 2009;461:91–4.
[9] Kang L, Wang J, Zhang Y, Kou Z, Gao S. IPS cells can support full-term development of tetraploid blastocyst-complemented embryos. Cell Stem Cell 2009;5:135–8.
[10] Zhao XY, Li W, Lv Z, Liu L, Tong M, Hai T, et al. IPS cells produce viable mice through tetraploid complementation. Nature 2009;461:86–90.
[11] Han J, Sidhu KS. Current concepts in reprogramming somatic cells to pluripotent state. Curr Stem Cell Res Ther 2008;3:66–74.
[12] Wu SM, Hochedlinger K. Harnessing the potential of induced pluripotent stem cells for regenerative medicine. Nat Cell Biol 2011;13:497–505.
[13] Buganim Y, Faddah DA, Cheng AW, Itskovich E, Markoulaki S, Ganz K, et al. Single-cell expression analyses during cellular reprogramming reveal an early stochastic and a late hierarchic phase. Cell 2012;150:1209–22.
[14] Soufi A, Donahue G, Zaret KS. Facilitators and impediments of the pluripotency reprogramming factors' initial engagement with the genome. Cell 2012;151:994–1004.
[15] Ebert AD, Yu J, Rose Jr FF, Mattis VB, Lorson CL, Thomson JA, et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 2009;457:277–80.
[16] Marchetto MC, Carromeu C, Acab A, Yu D, Yeo GW, Mu Y, et al. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. Cell 2010;143:527–39.
[17] Liu GH, Barkho BZ, Ruiz S, Diep D, Qu J, Yang SL, et al. Recapitulation of premature ageing with iPSCs from Hutchinson-Gilford progeria syndrome. Nature 2011;472:221–5.
[18] Zhang J, Lian Q, Zhu G, Zhou F, Sui L, Tan C, et al. A human iPSC model of Hutchinson Gilford Progeria reveals vascular smooth muscle and mesenchymal stem cell defects. Cell Stem Cell 2011;8:31–45.
[19] Liu GH, Qu J, Suzuki K, Nivet E, Li M, Montserrat N, et al. Progressive degeneration of human neural stem cells caused by pathogenic LRRK2. Nature 2012;491:603–7.
[20] Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flugel L, et al. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. N Engl J Med 2010;363:1397–409.
[21] Kim C, Wong J, Wen J, Wang S, Wang C, Spiering S, et al. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. Nature 2013;494:105–10.
[22] Schwartz RE, Trehan K, Andrus L, Sheahan TP, Ploss A, Duncan SA, et al. Modeling hepatitis C virus infection using human induced pluripotent stem cells. Proc Natl Acad Sci U S A 2012;109:2544–8.
[23] Trounson A, Shepard KA, DeWitt ND. Human disease modeling with induced pluripotent stem cells. Curr Opin Genet Dev 2012;22:509–16.
[24] Si-Tayeb K, Duclos-Vallee JC, Petit MA. Hepatocyte-like cells differentiated from human induced pluripotent stem cells (iHLCs) are permissive to hepatitis C virus (HCV) infection: HCV study gets personal. J Hepatol 2012;57:689–91.
[25] Sourisseau M, Goldman O, He W, Gori JL, Kiem HP, Gouon-Evans V, et al. Hepatic cells derived from induced pluripotent stem cells of pigtail macaques support hepatitis C virus infection. Gastroenterology 2013, PII: S0016-5085(13)01077-9.
[26] Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*. Nature 2011;470:105–9.
[27] Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 2013;499:481–4.
[28] Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science 2007;318:1920–3.
[29] Lu TY, Lin B, Kim J, Sullivan M, Tobita K, Salama G, et al. Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. Nat Commun 2013;4:2307.
[30] Schwartz SD, Hubschman JP, Heilwell G, Franco-Cardenas V, Pan CK, Ostrick RM, et al. Embryonic stem cell trials for macular degeneration: a preliminary report. Lancet 2012;379:713–20.
[31] Tong M, Lv Z, Liu L, Zhu H, Zheng QY, Zhao XY, et al. Mice generated from tetraploid complementation competent iPS cells show similar developmental features as those from ES cells but are prone to tumorigenesis. Cell Res 2011;21:1634–7.
[32] Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, Woltjen K. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature 2009;458:771–5.
[33] Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hamalainen R, et al. PiggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 2009;458:766–70.
[34] Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. Science 2008;322:949–53.
[35] Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. Science 2008;322:945–9.
[36] Gonzalez F, Barragan Monasterio M, Tiscornia G, Montserrat Pulido N, Vassena R, Batlle Morera L, et al. Generation of mouse-induced pluripotent stem cells by transient expression of a single nonviral polycistronic vector. Proc Natl Acad Sci U S A 2009;106:8918–22.
[37] Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science 2009;324:797–801.
[38] Zhou W, Freed CR. Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. Stem Cells 2009;27:2667–74.
[39] Wang Y, Chen J, Hu JL, Wei XX, Qin D, Gao J, et al. Reprogramming of mouse and human somatic cells by high-performance engineered factors. EMBO Rep 2011;12:373–8.
[40] Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. Nature 2011;474:212–5.
[41] Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell 2009;4:472–6.
[42] Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 2009;4:381–4.
[43] Hou P, Li Y, Zhang X, Liu C, Guan J, Li H, et al. Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science 2013;341:651–4.

[44] Gidekel S, Pizov G, Bergman Y, Pikarsky E. Oct-3/4 is a dose–dependent oncogenic fate determinant. Cancer Cell 2003;4:361–70.
[45] Hochedlinger K, Yamada Y, Beard C, Jaenisch R. Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. Cell 2005;121:465–77.
[46] Levings PP, McGarry SV, Currie TP, Nickerson DM, McClellan S, Ghivizzani SC, et al. Expression of an exogenous human Oct-4 promoter identifies tumor-initiating cells in osteosarcoma. Cancer Res 2009;69:5648–55.
[47] Bass AJ, Watanabe H, Mermel CH, Yu S, Perner S, Verhaak RG, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet 2009;41:1238–42.
[48] Rowland BD, Peeper DS. KLF4, p21 and context-dependent opposing forces in cancer. Nat Rev Cancer 2006;6:11–23.
[49] Pelengaris S, Khan M, Evan G. C-MYC: more than just a matter of life and death. Nat Rev Cancer 2002;2:764–76.
[50] Hart AH, Hartley L, Parker K, Ibrahim M, Looijenga LH, Pauchnik M, et al. The pluripotency homeobox gene NANOG is expressed in human germ cell tumors. Cancer 2005;104:2092–8.
[51] Piestun D, Kochupurakkal BS, Jacob-Hirsch J, Zeligson S, Koudritsky M, Domany E, et al. Nanog transforms NIH3T3 cells and targets cell-type restricted genes. Biochem Biophys Res Commun 2006;343:279–85.
[52] Lu X, Mazur SJ, Lin T, Appella E, Xu Y. The pluripotency factor nanog promotes breast cancer tumorigenesis and metastasis. Oncogene 2013. http://dx.doi.org/10.1038/onc.2013.209.
[53] Banito A, Gil J. Induced pluripotent stem cells and senescence: learning the biology to improve the technology. EMBO Rep 2010;11:353–9.
[54] Araki R, Uda M, Hoki Y, Sunayama M, Nakamura M, Ando S, et al. Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. Nature 2013;494:100–4.
[55] Guha P, Morgan JW, Mostoslavsky G, Rodrigues NP, Boyd AS. Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. Cell Stem Cell 2013;12:407–12.
[56] Chin MH, Mason MJ, Xie W, Volinia S, Singer M, Peterson C, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell 2009;5:111–23.
[57] Pasi CE, Dereli-Oz A, Negrini S, Friedli M, Fragola G, Lombardo A, et al. Genomic instability in induced stem cells. Cell Death Differ 2011;18:745–53.
[58] Laurent LC, Ulitsky I, Slavin I, Tran H, Schork A, Morey R, et al. Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. Cell Stem Cell 2011;8:106–18.
[59] Hussein SM, Batada NN, Vuoristo S, Ching RW, Autio R, Narva E, et al. Copy number variation and selection during reprogramming to pluripotency. Nature 2011;471:58–62.
[60] Gore A, Li Z, Fung HL, Young JE, Agarwal S, Antosiewicz-Bourget J, et al. Somatic coding mutations in human induced pluripotent stem cells. Nature 2011;471:63–7.
[61] Stiewe T. The p53 family in differentiation and tumorigenesis. Nat Rev Cancer 2007;7:165–8.
[62] Vousden KH, Lane DP. P53 in health and disease. Nat Rev Mol Cell Biol 2007;8:275–83.
[63] Meek DW. Tumour suppression by p53: a role for the DNA damage response? Nat Rev Cancer 2009;9:714–23.
[64] Zhao Y, Yin X, Qin H, Zhu F, Liu H, Yang W, et al. Two supporting factors greatly improve the efficiency of human iPSC generation. Cell Stem Cell 2008;3:475–9.
[65] Hong H, Takahashi K, Ichisaka T, Aoi T, Kanagawa O, Nakagawa M, et al. Suppression of induced pluripotent stem cell generation by the p53–p21 pathway. Nature 2009;460:1132–5.
[66] Kawamura T, Suzuki J, Wang YV, Menendez S, Morera LB, Raya A, et al. Linking the p53 tumour suppressor pathway to somatic cell reprogramming. Nature 2009;460:1140–4.
[67] Li H, Collado M, Villasante A, Strati K, Ortega S, Canamero M, et al. The Ink4/Arf locus is a barrier for iPS cell reprogramming. Nature 2009;460:1136–9.
[68] Marion RM, Strati K, Li H, Murga M, Blanco R, Ortega S, et al. A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. Nature 2009;460:1149–53.
[69] Utikal J, Polo JM, Stadtfeld M, Maherali N, Kulalert W, Walsh RM, et al. Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. Nature 2009;460:1145–8.