Special Issue: Genomic Era

Mini Review

Evaluation of safety of induced pluripotent stem cells by genome integrity

Akira Watanabe[1,2,*], Naoki Amano[1], Yumie Tokunaga[3], Unyanee Poolsap[1], and Shinya Yamanaka[1,2]

[1]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan
[2]The Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto, Japan
[3]Amelieff Co. Ltd., Kobe, Japan

The discovery of induced pluripotent stem (iPS) cells have opened the doors for further disease research, drug screening, as well as regenerative medicine. To achieve clinical application of iPS cells, it is important to select proper iPS cell lines that do not harbor the risk of tumorigenicity. Thus, it is desired to establish methodologies for evaluating the safety of iPS cells, particularly in terms of genome integrity. Massively parallel sequencing can be used to monitor genomic aberrations such as the subchromosomal and the single nucleotide variations. Refined mutation analyses of iPS and founder cells revealed that some of the iPS cell-specific variations were also detected in rare populations of the founder cells by consequence of capturing the heterogeneity of the founder cells. In this review, we highlight recent analyses used to evaluate the genome integrity of iPS cells, discuss future of directions for precise assessment of the safety of iPS cells, and address issues that should be overcome.

Rec.12/24/2013, Acc.2/18/2014, pp87-93

*Correspondence should be addressed to:
Akira Watanabe, Center for iPS Cell Research and Application, Kyoto University, 53, Shogoin-kawaharacho, Sakyo-ku, Kyoto-shi, Kyoto 606-8507, Japan. Phone: +81-75-366-7164, Fax, +81-75-366-7090, E-mail: a.watanabe@cira.kyoto-u.ac.jp

Key words DNA copy number variations, genomic heterogeneity, iPS cells, massively parallel sequencing, single nucleotide variations

Introduction

Induced pluripotent stem (iPS) cells are generated by the enforced expression of transcription factors, most commonly Oct3/4, Sox2, c-Myc and Klf4. In addition to pluripotency, iPS cells have infinite capacity for self-renewal[1-3]. The characteristics of fully reprogrammed cells are functionally and molecularly very similar to those of embryonic stem (ES) cells in terms of their morphologies, gene expression profiles, and capacities to differentiate into any of the following three germ layers: endoderm, mesoderm, and ectoderm. iPS cells could be a useful source for cell transplantation therapy, drug screening, and disease modeling[4, 5].

However, it is necessary to assess the risk of tumorigenicity of ES, iPS, and multipotent stem cells[6].

Several pioneering gene therapies using transduced hematopoietic cells showed aberrant activation of adjacent proto-oncogenes by retroviral vector insertion, resulting in formation of cancers such as leukemia[7-12]. The first generation of iPS cells was established by the vector-integration method, which may also cause genotoxicity via heritable and potentially toxic or deleterious effect on genomic DNA[3].

To avoid insertional genotoxicity in retroviral-induced iPS cells, non-integration delivery methods using plasmids, RNA, and proteins have been developed[13]. However, the insertional genotoxicity is not the only issue to be resolved. Several cell divisions with DNA replication during generation of iPS cells and their maintenance may expose cells to the risk of genomic aberrations, including DNA mutations and structural alterations. Such genomic aberrations of ES and iPS cells may not only affect tumorigenicity but also stem cell identity and differentiation capacity[14, 15]. The most appropriate assessment is *in vivo* experimentation by orthotopic injection of the cells into animal models. The mouse model is easily accessible, but it is difficult to approximate whether this model can recapture the environment of human tumors. A monkey model may be preferred for analogous assay to human tumor model; however, there are drawbacks such as low-throughput screening and ethical issues in the use of primates. Thus, instead of *in vivo* approaches, genomic analysis of iPS cells prior to clinical use is essential to assess the risk of tumorigenicity.

Recent advances in massively parallel sequencing have facilitated to rapidly obtain large amounts of sequencing data with simultaneous semi-terabase information. This sequencing has enabled high-resolution and -throughput assessment of DNA copy number variations (CNVs) and single nucleotide variations (SNVs), to identify a series of genetic diseases and other health-related traits[16]. For example, several consortia have identified a large number of cancer-specific mutations, including possible targets of anticancer drugs[17]. In addition, several studies have recently examined CNVs and SNVs of iPS cells using massively parallel sequencer[14, 15].

Aneuploidy and Subchromosomal Variations

CNVs may affect the properties of ES and iPS cells through perturbing gene expression profiles with altered gene dosages. A previous study identified one iPS cell clone with a distinctly different gene expression profile, proliferation rate, and differentiation potential to hematopoietic lineage compared with all other iPS cell lines[18]. Thus, it is important to select proper iPS cell lines that do not harbor hazardous CNVs. The International Stem Cell Initiative consortium addressed genomic alterations of pluripotent stem cells using 125 human ES and 11 iPS cell lines[19]. They reported that one-third of ES and iPS cell lines had karyotype abnormalities, whereas another study demonstrated that approximately 13% of ES and iPS cell lines had abnormal karyotypes[20]. This consortium and other studies have identified frequent duplications of chromosomes 12 and 20 in whole or in part, and less frequent trisomy of chromosomes 8 and X[19-21]. In addition, trisomy 17 has been frequently observed in human ES cells, but not in iPS cells[20, 21]. DNA copy number analysis of mouse iPS cells also detected CNVs at chromosome 11 that included conserved synteny regions with human chromosome 17, supporting the contention that human chromosome 17 is mechanically sensitive to DNA copy number alteration of pluripotent stem cells[22]. These observations imply that some selective pressures induce CNVs during reprogramming. The other studies reported that prolonged culture of the cells decreases the number and size of CNVs[23, 24] and indicated that prolonged culture exposed the cells to selection pressure. For example, CNV analysis of 22 iPS cell lines, three donor fibroblasts, and 17 ES cell lines has previously revealed that reprogrammed populations at very early passage had extensive genomic mosaicism with CNVs, and continued passage decreased the number and size of CNVs[23].

SNVs

The first study, which performed whole exome sequencing of 22 iPS cell lines, reported that approximately six SNVs in protein-coding regions were detected in each iPS cell line[25] and that their mutations were highly observed in proto-oncogenes registered in the Catalogue of Somatic Mutations in Cancer (COSMIC) database[26]. On the other hand, recent reports did not observe any significant enrichment of cancer-related mutations in iPS cell-specific SNVs[27, 28]. Because they used cord blood-derived iPS cell lines generated by episomal vector method, source of iPS cells and generation method may affect mutation event as

discussed below.

As well as genomic mosaicism with different CNVs[24, 29, 30], several studies reported that some of SNVs, which were detected only in iPS cells by rough exome sequencing, were also detected in rare populations of founder cells[25, 27, 30]. Therefore, it is important to clarify whether SNVs or CNVs detected by rough screening with massively parallel sequencing are truly iPS cell-specific or already existed in a rare population of the founder cells. Ji et al.[30] performed sequencing of the same iPS cell line at different passages and simulated a mutation rate of fibroblasts and iPS cells. This study demonstrated that *in vitro* passaging contributed 7% of the mutations in iPS cells and ultra-deep sequencing showed that 19% of the mutations were pre-existing in the parental fibroblasts, where the remaining 74% of the mutations were acquired during the reprogramming process. The observed increase in the number of mutations with prolonged culture and the mutation rate calculated in silico were consistent with an earlier report[25].

iPS Cell Captured the Heterogeneity of the Founder Cells

There is an increasing evidence that human somatic cells exhibit heterogeneity in genomic sequences[31, 32]. Although several previous studies reported iPS cell-specific CNVs[18, 19, 21, 23, 24, 29, 33, 34], the genetic mosaicism existing in the founder cells should be considered to identify true iPS cell-specific CNVs. Abyzov et al.[29] detected iPS cell-specific CNVs by whole-genome and transcriptome analyses of 20 human iPS cell lines and seven parental fibroblasts. However, by a more refined analysis using droplet digital PCR, at least 50% of those CNVs were also presented in small populations of parental fibroblasts from which each corresponding iPS cell line was derived[25]. They also suggested widespread mosaicism in human somatic cells by estimating that approximately 30% of fibroblasts had somatic CNVs. Thus, a comparison to the founder cells is essential to identify true iPS cell-specific CNVs.

It remains controversial whether iPS cell-specific SNVs and CNVs are consequence of selective pressure and also facilitate reprogramming. Several studies repored that the SNVs and CNVs observed only in iPS cells but not in the founder cells may be consequences of selective pressure during reprogramming[30, 35]. For example, mutation intensity during reprogramming was nine-fold higher than the background mutation rate in culture[30]. On the other hand, one reported that *de novo* mutations that could not be detected in the founder cells appeared to arise randomly during reprogramming because no common mutations were observed even among isogenic iPS cell lines[25]. Another study reported that iPS cell-specific SNVs in exonic regions were independent of the original cell type and thus supported the notion that mutation events occurred randomly[36]. And they could not observe any changes in efficiency of iPS cell generation even when genes with iPS cell-specific SNVs were silenced or over-expressed during reprogramming[36]. In addition, the traditional kernel density estimation to detect SNVs harbored in rare populations was used to monitor heterogeneity, but failed to identify shared SNVs in any clones in two of three experiments[31]. These findings supported that iPS cell-specific SNVs were unlikely to provide a selective advantage for reprogramming.

A comparative analyses for iPS cells using both reprogrammed cells and the corresponding fibroblasts or blood cells are feasible, however, it is difficult to do the same using human ES cell lines because they are produced by culturing inner cell masses of donated blastocysts and thus their genetic informationof the founder cells is generally inaccessible. In addition, although clones of iPS cells are generally derived from a single somatic cell, the ES cell line is composed of multiple subclones[37]. Thus, it is hard to identify ES cell-specific genomic variations.

Single-cell Analysis of SNVs and CNVs

Although conventional karyotyping by G-banding can detect chromosomal abnormalities at the single-cell level, the results are occasionally not reproducible because judgment of the karyotyping depends on the skill of observers and the relatively small number of cells (generally up to 100). Thus, objective examination of karyotyping, such as single cell analyses of SNVs and CNVs, is eagerly desired.

Single-cell sequencing for CNV and SNV analysis continues to rapidly progress. For example, multiple displacement amplification (MDA) followed by whole-genome sequencing has been applied for single-cell CNV detection. Navin et al.[38] were the first to report single-cell CNV analysis using breast cancer tissues and cell lines. They observed different patterns of CNVs in among each single cell from the same cancer tissues or cell lines, and proposed that, in contrast to gradual models of tumor progression, tumors grow by punctuated clonal expansion with few persistent intermediates. A second study reported the devel-

opment of a new amplification method composed of multiple annealing and looping-based amplification cycles[39]. This method was able to detect not only CNVs but also SNVs at the single-cell level. In addition, McConnell et al.[40] performed single cell CNV analysis of human neuronal cells by both single-nucleotide polymorphism (SNP) array-based analysis and whole-genome sequencing. They found that 13% to 41% of neuronal cells had *de novo* CNVs (>1Mb), indicating that CNVs were abundantly existed in human neurons.

Although SNV and CNV analyses at the single-cell level are supposedly the best methods for precise assessment of tumorigenic risk, these single-cell genome analyses are currently utilized only in pre-clinical trials. The sequence cost for such high-resolution SNV and CNV analyses using a portion of individual cells, for example with more than 100 single cells, is not reasonable at the present time. However, it is expected that the sequence cost will decrease for clinical use in the near future, as the cost performance of sequencing over the last decade has outpaced that predicted by Moore's law, which describes the computing industry's trend of doubling computer power every 2 years[41]. In addition, genotyping of two alleles from a single cell requires relatively lower sequence depth such as >10×, whereas that of bulk population generally requires a sequence depth of >30×.

Technical Concerns

Optimizing culture conditions and methods for iPS cell generation may reduce iPS cell-specific SNVs and CNVs. For example, the number of iPS cell-specific SNVs were decreased when *ZSCAN4*, which maintains genomic stability of pluripotent stem[42, 43] and reprograming cells[28, 44], was overexpressed. Cheng et al.[27] demonstrated that reprograming of human cord blood (CB) CD34$^+$ cells using episomal vectors was a preferable method for iPS cell generation. They could not detect any iPS cell-specific CNVs in three iPS cell lines even when cells cultured up to 51 passages[27], whereas others found iPS cell-specific CNVs in a part of iPS cell lines generated from fibroblast by integrating viral vectors[23, 24, 33]. Not only CNVs but also SNVs in CB-derived iPS cell lines was observed much less than those in iPS cells derived from fibroblasts or marrow stromal cells (MSCs)[27, 28]. One of the possibilities for less genomic alterations in CB-derived iPS cells is because parental CB CD34$^+$ cells are obtained after less expansion of the cells than adult fibroblasts. Another possibility is that CB CD34$^+$ cells are more homogenous population than fibroblasts that are highly exposed to environmental insults[45, 46].

The most considerable issue of sequence-based analysis is sequencing bias. High GC regions are difficult to sequence and highly repetitive sequences are hard to map to the human genome. The current sequencing technology cannot cover all exonic regions, even though sequencing with higher read depth broadens sequenceable exonic regions, however, further advancements in sequencing technology may overcome these issues. In addition to complete sequencing of exonic regions, it may be necessary to perform whole genome sequencing because extra-exonic SNVs may play a role in the efficiency of iPS cell generation, differentiation, and tumorigenicity.

For CNV analyses, sequence-based approaches may capture relatively small size of CNVs, which cannot be detected by SNP array-based analysis. This sequence-based strategy requires high sequence coverage in general, but a pipeline for single-cell CNV detection with low sequence coverage has been recently developed[47]. Moreover, improvements in the algorithms for the other structural alterations such as breakpoints of translocations are anticipated. For example, the progress in longer sequence reads will undoubtedly improve the efficiency of detecting such structural alterations.

There are other technical issues to overcome with these sequence-based analyses, particularly in bioinformatical analyses. Several algorithms to identify genomic variations using sequencing data have been developed, but there remain serious discrepancies among different pipelines for calling SNVs and small insertions/deletions[48]. Thus, improved accuracy of variation calling is eagerly desired, particularly when dealing with low sequence read depth from whole genome sequencing of a single cell. Refined analyses by amplicon-sequencing for target regions could be also helpful to increase the accuracy of these methods[30, 48].

Future Perspectives

There is a vast amount of information on cancer-associated mutations in the literatures and from large-scale screenings conducted by international consortia[17]. Comparisons of observed iPS cell-specific SNVs to mutations in the COSMIC database have been conducted to predict causal mutations linked to tumorigenesis[25, 27, 28]. However,

the list of deposited mutations is incomplete. The COSMIC database is composed of not only driver mutations but also resultant SNVs that do not contribute to tumor development or progression. In addition, as we discussed above, the list of cancer-associated genes may include SNVs arisen from clonal expansion of individual non-cancerous cells. And one cannot make conclusions before considering the functional contribution of SNVs to tumorigenesis because different histories of tumorigenesis exhibit diverse mutations.

The number and the position of SNVs and CNVs acquired during the reprogramming process and whether these variations affect the efficiency of reprogramming, tumorigenesis, or differentiation potential should be considered. Because the major concern for the clinical application of iPS cells is their propensity to form tumors, several studies assessed the risk of tumorigenesis by characteristics of the SNVs[49, 50]. It is unreasonable to perform functional assays for every iPS cell-specific genomic variation because it is labor intensive and time consuming. Rapid progress in computational approaches can be used to predict biological consequence of altered protein function due to amino acid substitutions[51]. Combinations of experimental evidence with *in silico* prediction would be helpful to predict hazardous genomic variations of iPS cells.

Besides surveys of SNVs by comprehensive analyses such as exome and whole-genome sequencing, detailed SNV analysis of cancer-related genes is important because very few cells in minor populations may harbor mutations that cause tumorigenesis. Ultra-deep sequencing of PCR amplicons can achieve a highly sensitive detection of SNVs, i.e., 0.01% existing in very small cell populations. iPS cells that present a risk of tumorigenesis could be ruled out by the ultra-deep sequencing of target regions such as previously reported proto-oncogenes and tumor suppressor genes.

Several previous studies focused on SNVs and CNVs of iPS cells, however, it is important to perform SNV and CNV analyses of differentiated cells that will be applied for transplantation. The risk of tumorigenicity of iPS cells for allo-transplantation as well as autologous transplantation should be also assessed by SNV and CNV analyses of iPS cells and their related founder cells. And genomic mosaicism of the iPS cell clone and iPS cell-derived differentiated cells should be evaluated because previous studies indicated 3 to 30 mutations per haploid genome were acquired in one mitotic division[27, 52].

In this review, we summarized recent reports on the genome integrity of iPS cells. It is critical to analyze CNVs and SNVs to examine the genome integrity of both iPS cells and related founder cells because it has been shown that some CNVs and SNVs observed only in iPS cells are consequences of cloning of the individual founder cells. Massively parallel sequencing technology is rapidly processing. Single-cell CNV and SNV analyses to assess the risk of genotoxicity of iPS cells for clinical applications are eagerly desired. Concomitantly with facilitating progress of sequencing technologies, improvement of *in silico* approaches including expanding and refining databases should be emphasized. The strategy for combining experimental evidences by sequencing and precise *in silico* prediction represents a basis of risk assessment of cell materials for regenerative medicine.

Acknowledgments and Source of Funding

We thank Masahiro Nakamura, Kazutoshi Takahashi, Keisuke Okita, Akiko Fukuhara, and Megumu Saito for excellent advice, and acknowledge Sachiko Ando and Yui Nomiya for sequencing. This study is supported in part by the grant of Funding Program for the World-Leading Innovative R&D on Science and Technology (FIRST), and Research Center Network for Realization of Regenerative Medicine JST-CREST program to S.Y., and CREST (JST) and Grant-in-Aid for Young Scientists (B) to A.W..

Conflict of Interest

S.Y. is a member without salary of the scientific advisory boards of iPierian, iPS Academia Japan, Megakaryon Corporation and HEALIOS K. K. Japan. Y.T. is an employee of Amelieff.

References

1) Takahashi K, Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006; 126: 663-676.
2) Yu J, Vodyanik MA, Smuga-Otto K, et al: Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007; 318: 1917-1920.
3) Takahashi K, Tanabe K, Ohnuki M, et al: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131: 861-872.
4) Takahashi K, Yamanaka S: Induced pluripotent stem cells in medicine and biology. Development. 2013; 140: 2457-2461.
5) Robinton DA, Daley GQ: The promise of induced pluripotent stem cells in research and therapy. Nature.

2012; 481: 295-305.

6) Cahan P, Daley GQ: Origins and implications of pluripotent stem cell variability and heterogeneity. Nat Rev Cell Biol. 2013; 14: 357-368.

7) Hacein-Bey-Abina S, Kalle Von C, Schmidt M, et al: LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science. 2003; 302: 415-419.

8) Ott MG, Schmidt M, Schwarzwaelder K, et al: Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. Nat Med. 2006; 12: 401-409.

9) Boztug K, Schmidt M, Schwarzer A, et al: Stem-cell gene therapy for the Wiskott-Aldrich syndrome. N Engl J Med. 2010; 363: 1918-1927.

10) Howe SJ, Mansour MR, Schwarzwaelder K, et al: Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest. 2008; 118: 3143-3150.

11) Seggewiss R, Pittaluga S, Adler RL, et al: Acute myeloid leukemia is associated with retroviral gene transfer to hematopoietic progenitor cells in a rhesus macaque. Blood. 2006; 107: 3865-3867.

12) Okita K, Ichisaka T, Yamanaka S: Generation of germline-competent induced pluripotent stem cells. Nature. 2007; 448: 313-317.

13) Okita K, Yamanaka S: Induced pluripotent stem cells: opportunities and challenges. Philos Trans R Soc Lond B Biol Sci. 2011; 366: 2198-2207.

14) Liang G, Zhang Y: Genetic and epigenetic variations in iPSCs: potential causes and implications for application. Cell Stem Cell. 2013; 13: 149-159.

15) Lund RJ, Närvä E, Lahesmaa R: Genetic and epigenetic stability of human pluripotent stem cells. Nat Rev Genet. 2012; 13: 732-744.

16) Ng SB, Buckingham KJ, Lee C, et al: Exome sequencing identifies the cause of a mendelian disorder. Nat Genet. 2010; 42: 30-35.

17) Kandoth C, McLellan MD, Vandin F, et al. Mutational landscape and significance across 12 major cancer types. Nature. 2013; 502: 333-339.

18) Mills JA, Wang K, Paluru P, et al: Clonal genetic and hematopoietic heterogeneity among human-induced pluripotent stem cell lines. Blood. 2013; 122: 2047-2051.

19) International Stem Cell Initiative: Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. Nat Biotechnol. 2011; 29: 1132-1144.

20) Taapken SM, Nisler BS, Newton MA, et al: Karotypic abnormalities in human induced pluripotent stem cells and embryonic stem cells. Nature Biotechnol. 2011; 29: 313-314.

21) Mayshar Y, Ben-David U, Lavon N, et al: Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. Cell Stem Cell. 2010; 7: 521-531.

22) Ben-David U, Benvenisty N: High prevalence of evolutionarily conserved and species-specific genomic aberrations in mouse pluripotent stem cells. Stem Cells. 2012; 30: 612-622.

23) Hussein SM, Batada NN, Vuoristo S, et al: Copy number variation and selection during reprogramming to pluripotency. Nature. 2011; 471: 58-62.

24) Martins-Taylor K, Nisler BS, Taapken SM, et al: Recurrent copy number variations in human induced pluripotent stem cells. Nat Biotechnol. 2011; 29: 488-491.

25) Gore A, Li Z, Fung H-L, et al: Somatic coding mutations in human induced pluripotent stem cells. Nature. 2011; 471: 63-67.

26) Forbes SA, Bindal N, Bamford S, et al: COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res. 2011; 39: D945-D950.

27) Cheng L, Hansen NF, Zhao L, et al: Low incidence of DNA sequence variation in human induced pluripotent stem cells generated by nonintegrating plasmid expression. Cell Stem Cell. 2012; 10: 337-344.

28) Su R-J, Yang Y, Neises A, et al: Few single nucleotide variations in exomes of human cord blood induced pluripotent stem cells. PLoS ONE. 2013; 8: e59908.

29) Abyzov A, Mariani J, Palejev D, et al: Somatic copy number mosaicism in human skin revealed by induced pluripotent stem cells. Nature. 2012; 492: 438-442.

30) Ji J, Ng SH, Sharma V, et al: Elevated coding mutation rate during the reprogramming of human somatic cells into induced pluripotent stem cells. Stem Cells. 2012; 30: 435-440.

31) Young MA, Larson DE, Sun C-W, et al: Background mutations in parental cells account for most of the ge-

netic heterogeneity of induced pluripotent stem cells. Cell Stem Cell. 2012; 10: 570-582.

32) Wang F, Pan X, Kalmbach K, et al: Robust measurement of telomere length in single cells. Proc Natl Acad Sci USA. 2013; 110: E1906-E1912.

33) Laurent LC, Ulitsky I, Slavin I, 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: 106-118.

34) Valamehr B, Abujarour R, Robinson M, et al: A novel platform to enable the high-throughput derivation and characterization of feeder-free human iPSCs. Sci Rep. 2012; 2: 213.

35) Hussein SMI, Elbaz J, Nagy AA: Genome damage in induced pluripotent stem cells: assessing the mechanisms and their consequences. Bioessays. 2013; 35: 152-162.

36) Ruiz S, Gore A, Li Z, et al: Analysis of protein-coding mutations in hiPSCs and their possible role during somatic cell reprogramming. Nat Commun. 2013; 4: 1382.

37) Humpherys D, Eggan K, Akutsu H, et al: Epigenetic instability in ES cells and cloned mice. Science. 2001; 293: 95-97.

38) Navin N, Kendall J, Troge J, et al: Tumour evolution inferred by single-cell sequencing. Nature. 2012; 472: 90-94.

39) Zong C, Lu S, Chapman AR, Xie XS: Genome-Wide Detection of Single-Nucleotide and Copy-Number Variations of a Single Human Cell. Science. 2012; 338: 1622-1626.

40) McConnell MJ, Lindberg MR, Brennand KJ, et al: Mosaic copy number variation in human neurons. Science. 2013; 342: 632-637.

41) Mardis ER. A decade's perspective on DNA sequencing technology. Nature. 2011; 470: 198-203.

42) Zalzman M, Falco G, Sharova LV, et al: Zscan4 regulates telomere elongation and genomic stability in ES cells. Nature. 2010; 464: 858-863.

43) Amano T, Hirata T, Falco G, et al: Zscan4 restores the developmental potency of embryonic stem cells. Nat Commun. 2013; 4: 1966.

44) Jiang J, Lv W, Ye X, et al: Zscan4 promotes genomic stability during reprogramming and dramatically improves the quality of iPS cells as demonstrated by tetraploid complementation. Cell Res. 2013; 23: 92-106.

45) Giorgetti A, Montserrat N, Aasen T, et al: Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. Cell Stem Cell. 2009; 5: 353-357.

46) Broxmeyer HE: Will iPS cells enhance therapeutic applicability of cord blood cells and banking? Cell Stem Cell. 2010; 6: 21-24.

47) Zhang C, Zhang C, Chen S, et al: A single cell level based method for copy number variation analysis by low coverage massively parallel sequencing. PLoS ONE. 2013; 8: e54236.

48) O'Rawe J, Jiang T, Sun G, et al: Low concordance of multiple variant-calling pipelines: practical implications for exome and genome sequencing. Genome Med. 2013; 5: 28.

49) Knoepfler PS: Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. Stem Cells. 2009; 27: 1050-1056.

50) Miura K, Okada Y, Aoi T, et al: Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol. 2009; 27: 743-745.

51) Ryan CJ, Cimermančič P, Szpiech ZA, Sali A, Hernandez RD, Krogan NJ: High-resolution network biology: connecting sequence with function. Nat Rev Genet. 2013; 14: 865-879.

52) Kinde I, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B: Detection and quantification of rare mutations with massively parallel sequencing. Proc Natl Acad Sci USA. 2011; 108: 9530-9535.