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Two-hit Reprograming of Induced Pluripotent Stem Cells

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Abstract

Endogenous and exogenous stresses produce reactive oxygen species (ROS) in cells, and these can cause DNA damage, apoptosis, autophagy, and senescence. The reprogramming of somatic cells to produce induced pluripotent stem cells (iPSCs) initially requires ROS production and then represses the endogenous expression of tumor suppressor factors such as p^{53}, $p21^{Cip1}$, and $p^{16Ink4a}$. This article discusses a two-hit model of stress-induced reprogramming for generating iPSCs and suggests new clinical tools for stem cell therapy.

Keywords: Environmental stress; Induced pluripotent stem cells; Stem cells; Stress; Reactive oxygen species; Reprograming

Induced Pluripotent Stem Cells

The aging of many modern societies is associated with increases in the rates of cancers, heart diseases, and chronic and age-related brain and mental diseases. The availability of adequate cell sources to repopulate injured or degenerated tissues is a central priority in regenerative medicine, and stem cells are invaluable candidates because of their capacity for self-renewal and ability to differentiate into several cell types [1]. Various stem cell types, including embryonic, fetal, perinatal, and adult stem cells and induced pluripotent stem cells (iPSCs), have been investigated as sources for regenerative therapies [2,3]. Although the extensive studies of iPSCs under laboratory conditions have been done, several problems should be solved for clinical setting of stem cells and iPSCs. However, the generation of iPSCs by reprogramming technology has provided the regenerative medicine field with new tools for cell replacement strategies and the modeling of human diseases. It has also stimulated the development of new drugs and enabled the screening of environmental disruptors and natural compounds that are potentially hazardous to human health. The delivery and expression of genetic reprogramming factors, the genomic instability and epigenetic memory if iPSCs, the impact of cell propagation in culture, the development of new drugs for disease, and the impact of environmental toxicants are only some of the concerns surrounding this technology.

Environmental Stress Factors

Many risk factors, such as air pollution, fuel exhaust emissions, tabacco smoking, polycyclic aromatic hydrocarbons, volatile organic chemicals, environmentally disruptive chemicals (EDCs), and mental stresses, have been suggested as culprits in triggering or exacerbating human diseases [4]. This has been exemplified clearly by a recent meta-analysis of the impact of residential environmental chemicals on the occurrence primarily of allergic and immunological diseases [5]. Establishing the extent to which particular EDCs might influence genomic function is an essential first step in defining their potential effects on the long-term viability of the target organism [6]. If an environmental disruptor can induce an epigenetic change that is heritable through mitosis, there is a potential for significant phenotypic effects long after the initiating factor has disappeared. Furthermore, if such mitotically heritable changes are induced in germ cells, then the transmission through meiosis to succeeding generations might be possible. Thus, the studies to examine the effects of EDCs on stem cells are crucial for understanding heritable risk factors.

Pre-iPSCs and Full-iPSCs

Reactive oxygen species (ROS), such as superoxide and hydroxyl radicals, are highly reactive and can damage mitochondrial and nuclear DNA, as well as proteins and lipids, by modifying them via oxidative reactions. Stem cells appear to be particularly sensitive to elevated ROS levels. Increased levels of ROS induced by metabolic changes in iPSCs might hinder the survival of reprogrammed cells [7]. In addition, mitochondrial functions are also repressed in iPSCs or human embryonic stem cells (ESCs) [8], suggesting that ROS generation by reprograming factors is unfavorable to the generation of iPSCs. In addition, it was reported that cell senescence impairs reprograming to iPSCs, and that reprograming triggers a stress response to senescence at the initial stage [9]. In fact, senescence is the irreversible arrest during the G1 phase of the cell cycle that is elicited by replicative exhaustion or in response to stresses such as DNA damage, drugs, or oncogenes. Moreover, oxidative stress also induces cellular apoptosis and autophagy. These effects occur primarily through activation of the tumor suppressor factor p53 and upregulation of the cyclin-dependent kinase (CDK) inhibitors $p16^{Ink4a}$ and $p21^{Cip1}$ [10]. The introduction of Yamanaka factors (*Oct4, Sox2, Klf4 and c-Myc*) initially triggers stress responses with characteristics of oxidative-stress-like increases in the levels of oxidized 8-oxoguanine and in reprograming-induced senescence (RIS) by upregulating p53, $p16^{Ink4a}$, and $p21^{Cip1}$ at the initial stage leading to pre-induced pluripotent stem cells (Pre-iPSCs). This upregulation of $p16^{Ink4a}$ and $p21^{Cip1}$ was observed during heterokaryon-based reprograming, suggesting the existence of an inherent link between senescence and reprograming. Subsequently, the elevated levels of $p16^{Ink4a}$ and $p21^{Cip1}$ that could be detected in Pre-iPSCs were decreased at a later stage in mouse embryonic fibroblasts (MEFs) [9,11]. The inhibition of senescence using gene knockdown constructs of p^{53}, $p21^{Cip1}$, and $p16^{Ink4a}$ at a late stage finally improved the efficiency of reprograming

of somatic cells or primary cancer cells, and the resulting iPSCs displayed characteristics of fully pluripotent stem cells (Full-iPSCs) [12]. Pre-iPSCs that failed to reprogram fully were trapped in a late step of reprograming [9,11]. Inhibition of DNA methylation, knockdown of lineage-specific genes, or treatment with two inhibitors [13] could either convert some of these Pre-iPSCs to Full-iPSCs or increase the proportion of fully reprogrammed iPSCs vs. Pre-iPSCs. The inhibition or alleviation of senescence can increase the number of cells that surpass the early barrier imposed by RIS, resulting in a higher number of both Pre-iPSCs and fully reprogrammed iPSCs. This RIS and probably also reprograming-induced apoptosis (RIA) act as initial barrier, limiting the efficiency of reprograming and making it slower and intermittent. To increase the efficiency of reprograming, the repression of RIS or RIA is definitely required at the late stage, followed by a decrease in the expression of $p^{16Ink4a}$, p^{21Cip1}, and p^{53} by the hypoxia condition or other conditions, which are necessary for full reprograming [14]. Thus, reprograming requires two stages. The initial stage includes ROS production induced by reprograming factors, which leads to the reprograming changes or DNA damage that in turn induce the expression of $p^{16Ink4a}$, p^{21Cip1}, and p^{53}. At this late stage, these alterations should be shut down by a reduction of the expression of p^{53}, p^{21Cip1}, and $p^{16Ink4a}$ by hypoxic conditions or the expression of stemness genes such as Oct^4, Sox^2, Nanog, Esrrb, $Tfcp^2l^1$ or other genes enhancing pluripotency. Of cause, we cannot rule out other hypotheses such as stochastic mechanism [15], link with innate immunity [16] and so on.

Two-hit Theory for Reprogramming of iPSC

To test this hypothesis, I propose forcing the expression of the AhR gene, encoding the arylhydrocarbon receptor, at the initial stage of reprograming. Subsequently, reduced ROS production and enhanced expression of the Nrf^2 gene, encoding nuclear factor (erythroid-derived)-like 2 (Nrf^2), should be combined in cells using conditional knockout and conditional forced expression systems at the second stage. The target-gene family of Nrf^2 is probably overlapped and compensate with tumor suppressor gene products such as p^{53}, p^{21Cip1}, and $p^{16Ink4a}$ for each other. The generation of iPSCs will be examined based on the expression of stemness marker genes and enzyme activity, as well as teratoma formation. DNA repair plays a critical role in the maintenance of genome stability. It senses any tiny or global genomic abnormality and quickly launches signaling to recruit DNA repair factors to the lesions, arrest cell cycle progression, or induce apoptosis. These diverse cellular responses are collectively called the DNA damage response (DDR) [17]. In addition to DNA repair, the spindle assembly checkpoint prevents errors in mitosis and contributes to genome stability. Because DNA repair prevents chromatin change, which occurs during reprograming, it also suppresses the generation of iPSC. We plan to investigate the roles of AhR and Nrf2 in reprograming, including the modulation of reprograming by intervening with DNA repair and DDR to improve success rates in iPSC generation. However, Shigeta et al. reported that the requirement of Trp^{53} in mice for inducing and maintaining the pluripotency of ESCs in-vivo is not absolute [18]. Thus, this indicates that another mechanism besides p^{53} and p^{21Cip1} might perform a compensatory function during reprograming.

Environmental risks and stem cells therapies

Drug discovery and development as well as drug screening to date have relied on animal models, which are useful but are often fail to mimic human physiology. The discovery of human iPSCs has led to the emergence of a new paradigm for drug screening using human-organ-like and disease-specific cultures in-vitro. Organ-like structures cultured in advanced microfluidic systems can simulate tissue structure and function at a microscope level and can enable high-throughput testing of different compounds for therapeutic and diagnostic application. The preliminary use of testicular iPSCs for testing the effect of environmental hormones such as phthalate derivatives has been reported [19]. We will utilize these technologies for the screening and development of medical drugs and natural compounds as well as novel small molecules for the future development of stem cell therapy.

Conclusion

The generation of iPSCs obtained by genomic reprograming technology has provided regenerative medicine with new tools for cell replacement strategies, the modeling of human diseases, development of new drugs, and screening of EDCs. The advantages of these pluripotent cells compared with other sources of stem cells include the generation of patient-derived cells and the lack of embryonic tissues by maintaining a versatile differentiation potential. Many challenges are yet to be circumvented before this technology can be translated widely to clinical settings. A large-scale screening of chemical libraries with patient-specific iPSCs or disease-specific iPSCs is currently underway, and is expected to lead to new drug discoveries. The effects of EDCs on stem cells are also being investigated using these strategies. Patient- or disease-specific iPSCs could be generated by ROS regulation via AhR, and by using the Nrf^2 cascade system for antioxidation. Theses should prove useful for preclinical studies of stem cell therapy.

References

1. Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. Nature 292: 154-156.

2. Wilmut I (2011) Consternation and confusion following EU patent judgment. Cell Stem Cell 9: 498-499.

3. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663-676.

4. Kavlock RJ, Ankley GT (1996) A perspective on the risk assessment process for endocrine-disruptive effects on wildlife and human health. Risk Anal 16: 731-739.

5. Chalubinski M, Kowalski ML (2006) Endocrine disrupters--potential modulators of the immune system and allergic response. Allergy 61: 1326-1335.

6. Fang L, Wuputra K, Chen D, Li H, Huang S-K et al. (2014) Environmental-stress-induced chromatin regulation and its heritability. J Carcinogenesis & Mutagenesis 5: 156.

7. Yoshida Y, Takahashi K, Okita K, Ichisaka T, Yamanaka S (2009) Hypoxia enhances the generation of induced pluripotent stem cells. Cell Stem Cell 5: 237-241.

8. Mah N, Wang Y, Liao MC, Prigione A, Jozefczuk J, et al. (2011) Molecular insights into reprogramming-initiation events mediated by the OSKM gene regulatory network. PLoS One 6: e24351.

9. Banito A, Rashid ST, Acosta JC, Li S, Pereira CF, et al. (2009) Senescence impairs successful reprogramming to pluripotent stem cells. Genes Dev 23: 2134-2139.

10. Collado M, Blasco MA, Serrano M (2007) Cellular senescence in cancer and aging. Cell 130: 223-233.

11. Mikkelsen TS, Hanna J, Zhang X, Ku M, Wernig M, et al. (2008) Dissecting direct reprogramming through integrative genomic analysis. Nature 454: 49-55.

12. Molchadsky A, Rivlin N, Brosh R, Rotter V, Sarig R (2010) p53 is balancing development, differentiation and de-differentiation to assure cancer prevention. Carcinogenesis 31: 1501-1508.

13. Silva J, Barrandon O, Nichols J, Kawaguchi J, Theunissen TW, et al. (2008) Promotion of reprogramming to ground state pluripotency by signal inhibition. PLoS Biol 6: e253.

14. Yamanaka S (2007) Strategies and new developments in the generation of patient-specific pluripotent stem cells. Cell Stem Cell 1: 39-49.

15. Hanna J, Saha K, Pando B, van Zon J, Lengner CJ, et al. (2009) Direct cell reprograming is a stochastic process amenable to acceleration. Nature 462: 595-601.

16. Lee J, Sayed N, Hunter A, Au KF, Wong WH, et al. (2012) Activation of innate immunity is required for efficient nuclear reprogramming. Cell 151: 547-558.

17. Lin YC, Murayama Y, Hashimoto K, Nakamura Y, Lin CS, et al. (2014) Role od tumor suppressor genes in the cabcer-associated reprogramming of human induced opluripotent stem cells. Stem Cell Res Ther 5: 58.

18. Wang SW, Wang SS, Wu DC, Lin YC, Ku CC, et al. (2013) Androgen receptor-mediated apoptosis in bovine testicular induced pluripotent stem cells in response to phthalate esters. Cell Death Dis 4: e907.

19. Shigeta M, Ohtsuka S, Nishikawa-Torikai S, Yamane M, Fujii S, et al. (2013) Maintenance of pluripotency in mouse ES cells without Trp53. Sci Rep 3: 2944.