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MINI-REVIEW

The genomic stability of induced pluripotent stem cells

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ABSTRACT

With their capability to undergo unlimited self-renewal and to differentiate into all cell types in the body, induced pluripotent stem cells (iPSCs), reprogrammed from somatic cells of human patients with defined factors, hold promise for regenerative medicine because they can provide a renewable source of autologous cells for cell therapy without the concern for immune rejection. In addition, iPSCs provide a unique opportunity to model human diseases with complex genetic traits, and a panel of human diseases have been successfully modeled *in vitro* by patient-specific iPSCs. Despite these progresses, recent studies have raised the concern for genetic and epigenetic abnormalities of iPSCs that could contribute to the immunogenicity of some cells differentiated from iPSCs. The oncogenic potential of iPSCs is further underscored by the findings that the critical tumor suppressor p53, known as the guardian of the genome, suppresses induced pluripotency. Therefore, the clinic application of iPSCs will require the optimization of the reprogramming technology to minimize the genetic and epigenetic abnormalities associated with induced pluripotency.

KEYWORDS induced pluripotent stem cells, reprogramming, genetic and epigenetic abnormalities

INTRODUCTION

The successful establishment of human embryonic stem cells (hESCs) could provide a renewable source of various cell types for human cell therapy (Thomson et al., 1998). Significant progress has been made in establishing conditions to differentiate hESCs into various lineages of therapeutically valuable cells (Fu and Xu, 2011). In addition, the ongoing clinical trial of hESC-based therapy of spinal cord injury and macular degeneration has further improved the feasibility of hESC-based cell therapy. While highly promising, there are several challenges facing hESC-based therapy, such as the ethical issues of destroying the human embryo and the immune rejection of the allogenic hESC-derived cells by the recipients (Fu and Xu, 2011). These challenges are overcome by the groundbreaking discovery of induced pluripotent stem cells (iPSCs) reprogrammed from somatic cells with defined factors (Oct4, Sox2, Klf4 and c-Myc) (Takahashi and Yamanaka, 2006). Soon after the initial discovery, scientists have been able to reprogram somatic cells including terminally differentiated cells from many species including human into iPSCs (Takahashi et al., 2007; Yu et al., 2007; Aasen et al., 2008; Aoi et al., 2008; Eminli et al., 2008; Hanna et al., 2008; Kim et al., 2008; Liu et al., 2008; Maherali et al., 2008; Park et al., 2008b; Stadtfeld et al., 2008; Eminli et al., 2009; Esteban et al., 2009; Li et al., 2009b; Utikal et al., 2009a; Wu et al., 2010). To conclusively demonstrate that iPSCs are equivalent to embryonic stem cells (ESCs) in the context of pluripotency, three independent groups successfully generated the iPSC-mice using tetraploid complementation (Boland et al., 2009; Kang et al., 2009; Zhao et al., 2009). Therefore, patient-specific iPSCs hold great potential to bypass the ethical controversies and immune rejection problem associated with hESCs in regenerative medicine.

IPSCS IN DISEASE MODELING

Mouse models for human diseases have been a powerful tool to help us understand the mechanism of pathogenesis in human diseases (Bedell et al., 1997a, 1997b). However, mouse models have limitations in studying human diseases due to the species-specific differences and inaccurate recapitulation of human disease phenotypes. In support of this

notion, most drugs that have worked well in mouse models fail in human clinical trials (Tiscornia et al., 2011). hESCs genetically modified with genetic mutations linked to human diseases provide an unlimited resource of various cell types affected in human diseases for mechanistic studies and drug discovery, thus opening up a new area of human disease modeling (Song et al., 2010). However, hESCs cannot be used to model human diseases with complex or unknown genetic traits, a challenge that can be overcome by iPSCs. In this context, iPSC technology has been used to model a large panel of human diseases including spinal muscular atrophy (Ebert et al., 2009), amyotrophic lateral sclerosis (Dimos et al., 2008), familial dysautonomia (Lee et al., 2009), Long QT syndrome (Moretti et al., 2010; Itzhaki et al., 2011), LEOPARD syndrome (Carvajal-Vergara et al., 2010), dyskeratosis congenital (Agarwal et al., 2010), Rett's syndrome (Marchetto et al., 2010), Timothy syndrome (Yazawa et al., 2011), schizophrenia (Brennand et al., 2011), Parkinson's disease (PD) (Nguyen et al., 2011), Hutchinson-Gilford progeria syndrome (Liu et al., 2011; Zhang et al., 2011), Alzheimer's disease (AD) (Israel et al., 2012) as well as adenosine deaminase deficiency-related severe combined immunodeficiency, Shwachman-Bodian-Diamond syndrome, Gaucher disease type III, Duchenne and Becker muscular dystrophy, Huntington disease, juvenile-onset, type 1 diabetes mellitus, Down syndrome/trisomy 21 and the carrier state of Lesch-Nyhan syndrome (Park et al., 2008a). In these human diseases such as type I diabetes, AD and PD, the limited access to the affected tissues and the inability to grow the affected cells in culture have hindered the development of effective treatment for these devastating diseases. The generation of disease-specific iPSCs will greatly facilitate the mechanistic studies and the development of therapeutic interventions.

REPROGRAMMING FACTORS: PLURIPOTENCY AND TUMORIGENICITY

The reprogramming factors include Oct4, Sox2, Nanog, Klf4, c-Myc and Lin28 (Takahashi et al., 2007; Yu et al., 2007). Oct4, Sox2 and Nanog are highly expressed in pluripotent lineages of the early embryo and play important roles in maintaining pluripotency of ESCs (Pesce and Schöler, 2001; Avilion et al., 2003; Chambers et al., 2003; Mitsui et al., 2003). The Oct4 protein levels are tightly regulated for the maintenance of pluripotent stem cells (Niwa et al., 2000). Sox2 can heterodimerize with Oct4 and is important for Oct4 dependent gene expression to stabilize the pluripotent state of ESCs (Masui et al., 2007). Genome-wide location analyses have shown that Oct4, Sox2 and Nanog all bind to their own promoters as well as each other's promoters, indicating a complex autoregulatory circuitry (Boyer et al., 2005; Loh et al., 2006). Klf4 and c-Myc are involved in a wide range of cellular processes, including proliferation, differentiation and cell growth (Dang et al., 2000; Dang et al., 2006). A possible role of Klf4 and c-Myc in reprogramming is to convert somatic cells into highly proliferative state associated with pluripotent stem cells (Yamanaka, 2007). Lin28 is shown to block the processing of the let-7 family miRNAs in ESCs that induces the differentiation (Newman et al., 2008; Viswanathan et al., 2008). Therefore, Lin28 may facilitate the reprogramming process by blocking the miRNA-mediated differentiation.

Mice generated with iPSCs reprogrammed by retroviral vectors developed malignant tumors, raising the concerns of the cancer risk of iPSCs (Okita et al., 2007; Tong et al., 2011). The spontaneous reactivation of C-myc could play an important role in inducing tumorigenesis. Although new methods have been developed to generate iPSCs in the absence of C-myc or without any random integration of the reprogramming factors, concerns for cancer risk remain because the other reprogramming factors also have oncogenic potential. For example, Oct4 has been implicated in tumor formation (Gidekel et al., 2003; Hochedlinger et al., 2005; Levings et al., 2009). Sox2 is overexpressed in many human cancers and is a lineage-specific oncogene (Bass et al., 2009). Klf4 functions as an oncogene in a context dependent manner (Rowland and Peeper, 2006). Nanog is overexpressed in many human cancers and might play a role in metastasis (Hart et al., 2005; Piestun et al., 2006; Chiou et al., 2010). Recent studies have also suggested that Lin28 can promote the cellular transformation and is associated with malignancies in multiple tumor types (Viswanathan et al., 2009; Peng et al., 2010).

To reduce the oncogenic potential, significant progress has been achieved by developing small molecule compounds that can improve the reprogramming efficiency or replace the reprogramming factors. For example, the reprogramming efficiency was significantly enhanced by small molecule compounds that are epigenetic modifiers including DNA methyltransferase inhibitor, histone methyltransferase inhibitor, histone demethylase inhibitor, histone deacetylase inhibitor, lysine-specific demethylase 1 inhibitor, and TGF-β pathway antagonist, MAPK/ERK inhibitor, PDK1 kinase activator, GSK3 inhibitor as well as vitamin C (Huangfu et al., 2008; Shi et al., 2008; Ichida et al., 2009; Li et al., 2009c; Lin et al., 2009; Esteban et al., 2010; Zhu et al., 2010; Li et al., 2011; Yuan et al., 2011). In addition, the reprogramming efficiency of the progenitor cells such as the hematopoietic stem cells and neural stem cells is greatly increased, while the requirement for the reprogramming factors is reduced, suggesting that the oncogenic potential of the induced pluripotency could be reduced when using the progenitor cells (Eminli et al., 2009). While promising, the oncogenic potential of iPSCs reprogrammed with these approaches remains to be evaluated.

P53 AND GENOMIC INSTABILITY OF IPSCS

As a tumor suppressor, p53 is critical to maintain genomic

stability in mammalian cells (Zhao and Xu, 2010). In response to DNA damage, p53 is activated to initiate cell cycle arrest and DNA damage repair process. Catastrophic damage will trigger the p53 to eliminate the damaged cell via p53 dependent senescence or apoptosis pathways (Ko and Prives, 1996). As the guardian of the genome, p53 plays an important role in maintaining the genetic stability in response to oncogenic stress. Consistent with the oncogenic potential of iPSCs, a series of studies have shown that p53 significantly suppresses the induced reprogramming (Zhao et al., 2008; Banito et al., 2009; Hong et al., 2009; Kawamura et al., 2009; Li et al., 2009a; Utikal et al., 2009b). In further support of this role of p53, published studies have shown that p53 maintains the genetic stability of the self-renewing embryonic stem (ES) cells by suppressing the expression of Nanog, leading to the differentiation of DNA-damaged ES cells (Lin et al., 2005). Therefore, the inactivation of p53 appears to be required for successful reprogramming, raising the concerns about the genetic instability of iPSCs and derivatives (Zhao and Xu, 2010). In support of this notion, the reprogramming is associated with DNA damage and the efficiency enhanced by p53 inhibition is directly proportional to the accelerated proliferation rate with increased DNA damage (Hanna et al., 2009; Lake et al., 2012), leading to iPSCs with persistent DNA damage and chromosomal aberrations (Marión et al., 2009). In addition, the p53 mutation has also been shown to augment the malignant potential of the reprogrammed cells (Sarig et al., 2010). In summary, these studies indicate that functional p53 is critical to ensure genetic stability during the reprogramming process. Instead of silencing p53 to increase the reprogramming efficiency at the expense of genetic stability, a recent study has demonstrated that the deficiency of Puma and p21 can increase the reprogramming efficiency to the same level as p53 deficiency, while prevent the accumulation of DNA damage during reprogramming (Lake et al., 2012). In this context, Puma-deficiency promotes the senescence pathway to eliminate the reprogramming cells with increased DNA damage. Therefore, this finding indicates the feasibility to increase the reprogramming efficiency without sacrificing the genomic integrity.

EPIGENETIC, GENETIC ABNORMALITY AND IMMUNOGENICITY OF IPSCS

The reprogramming of somatic cells into iPSCs involves the re-establishment of ESC-like epigenetics. Although the majority of the epigenome of iPSCs are similar to that of ESCs, there remains a significant difference including the aberrant silencing of imprinted genes and DNA methylation patterns such as the residual epigenetic memories from somatic cells of origin (Pick et al., 2009; Kim et al., 2010; Polo et al., 2010; Stadtfeld et al., 2010; Lister et al., 2011). Furthermore, some epigenetic abnormalities of iPSCs are common among iPSCs from different origins of somatic cells, indicating that some of the epigenetic abnormalities of the iPSCs are induced during the reprogramming (Lister et al., 2011).

In addition to the epigenetic abnormalities, recent studies have identified genomic abnormalities such as chromosomal aneuploidy and translocations, megabase-scale duplications and deletions, and point mutations in iPSCs (Mayshar et al., 2010; Gore et al., 2011; Hussein et al., 2011; Laurent et al., 2011). Gore and colleagues have identified that point mutations have been accumulated particularly in oncogenic pathways in otherwise karyotypically normal human iPSCs (Gore et al., 2011). Hussein and colleagues have reported that early-passage human iPSCs contained increased copy number variants (CNVs) compared to intermediate-passage iPSCs cells or ESCs (Hussein et al., 2011). Some of these aberrations show a high incidence of chromosome 12 duplications, resulting in upregulation of Nanog and Gdf3, which may facilitate the adaptation process during reprogramming (Mayshar et al., 2010). Laurent and colleagues found increased subchromosomal CNVs in pluripotent cell samples with the enriched CNVs located in specific genomic regions (Laurent et al., 2011). They also found increased numbers of deletions in human iPSCs samples associated with tumor-suppressor genes, whereas duplications of oncogenes are found in iPSCs that have been cultured for extended time (Laurent et al., 2011).

ESCs can undergo unlimited self-renewal and retain the pluripotency to differentiate into all cell types in the body, and thus hold great promise for cell replacement therapy. However, one major obstacle is that the cells derived from established human ESC lines are allogeneic and immune rejected by the recipients. The patient-specific iPSCs could mitigate this problem as a renewable source of autologous cells for human therapy. Although it has been widely assumed that the autologous cells derived from patient-specific iPSCs are immune tolerant in that patient, recent studies have shown that the derivatives of mouse iPSCs can be immunogenic in syngeneic recipients (Zhao et al., 2011). Global gene expression analysis of teratomas formed by B6 ESCs and iPSCs revealed a number of genes overexpressed in teratomas derived from iPSCs, and several of these genes' products directly contributed to the immunogenicity of the B6 iPSCs-derived cells in B6 mice in a T cell dependent manner. While remained to be determined, the aberrant expression of these minor antigens could be due to the epigenetic difference between iPSCs and ESCs. In addition, the contribution of genetic mutations to the immunogenicity of iPSC derivatives remains to be evaluated.

CONCLUSION REMARKS

The groundbreaking discovery of iPSCs has reshaped the scientific and political landscapes of stem cell biology. It provides an unprecedented opportunity to model human disease and re-examine some of the basic biology such as develop-

ment and differentiation. The potential of iPSCs in drug discovery is tremendous. However, recent findings of epigenetic and genetic abnormalities in iPSCs raise the safety concerns of iPSCs in human cell therapy. In addition, the cancer risk associated with induced pluripotency must be vigorously addressed before any clinical application of iPSCs.

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