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REVIEW

Clinical potential of human-induced pluripotent stem cells

Perspectives of induced pluripotent stem cells

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Abstract The recent establishment of induced pluripotent stem (iPS) cells promises the development of autologous cell therapies for degenerative diseases, without the ethical concerns associated with human embryonic stem (ES) cells. Initially, iPS cells were generated by retroviral transduction of somatic cells with core reprogramming genes. To avoid potential genotoxic effects associated with retroviral transfection, more recently, alternative non-viral gene transfer approaches were developed. Before a potential clinical application of iPS cell-derived therapies can be planned, it must be ensured that the reprogramming to pluripotency is not associated with genome mutagenesis or epigenetic aberrations. This may include direct effects of the reprogramming method or "off-target" effects associated with the reprogramming or the culture conditions. Thus, a rigorous safety testing of iPS or iPS-derived cells is imperative, including long-term studies in model animals. This will include not only rodents but also larger mammalian model species to allow for assessing long-term stability of the transplanted cells, functional integration into the host tissue, and freedom from undifferentiated iPS cells. Determination of the necessary cell dose is also critical; it is assumed that a minimum of 1 billion transplantable cells is required to achieve a therapeutic effect. This will request medium to long-term in vitro cultivation and dozens of cell divisions, bearing the risk of accumulating replication errors. Here, we review the clinical potential of human iPS cells and evaluate which are the most suitable approaches to overcome or minimize risks associated with the application of iPS cell-derived cell therapies.

Keywords Cell fate · Cellular reprogramming · Cell therapy · Genotoxicity · Integrational mutagenesis · Ontogenesis · Transposition

Abbreviations

ALS	Amyotrophic lateral sclerosis
AMD	Age-related macular degeneration
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats, CRISPR-associated protein 9
ERK1/ERK2	Extracellular signal-regulated kinases 1 and 2
ES	Embryonic stem (cell)
FRM1	X-linked fragile X mental retardation 1
GMP	Good manufacturing practice
GSK3ß	Glycogen synthase kinase 3 beta
HLA	Human leukocyte antigen
HD	Huntington's disease

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iPS	Induced pluripotent stem (cell)
NHP	Non-human primate
pSC	Parthenogenetic stem cell
RPE	Retinal pigment epithelium
SCID	Severe combined immunodeficiency
SSEA-5	Stage-specific embryonic antigen-5
TALEN	Transcription activator-like effector nuclease

Introduction

Pluripotent stem cells, such as human embryonic stem (hES) cells, human parthenogenetic stem cells (pSCs), or induced pluripotent stem (iPS) cells, are able to differentiate into any cell type of the body; thus, they represent a basic tool for innovative cell therapies. Human ES cells have been derived from cells of the pre-implantation embryo (Thomson et al. 1998). Therapeutic cloning represents a deviation of this methodology by the transfer of a cell nucleus isolated from a somatic cell into an enucleated oocyte, activation and culture of the reconstructed embryo to the blastocyst stage, followed by the derivation of cloned ES cells from the inner cell mass (Ma et al. 2014). However, the derivation of ES cells via the destruction of a human embryo, resulting from fertilization or cloning, for the potential sake of a patient, is ethically controversially discussed and has tainted the human ES cell field (de Miguel-Beriain 2015; Trounson and DeWitt 2016).

The derivation of human pluripotent cells from parthenogenetic embryos seems to be less controversial. Parthenogenetic SCs have been created by chemically stimulating unfertilized oocytes to initiate cleavage divisions. Different activation techniques allowed the creation of either human leukocyte antigen (HLA) heterozygous pSCs or HLA homozygous pSCs (Drukker 2008; Espejel et al. 2014; Schmitt et al. 2015). However, it is not clear whether the loss of epigenetic imprinting may limit the clinical usefulness of pSCs (Hernandez et al. 2003).

The seminal discovery of iPS cells offers an elegant alternative to the embryo or oocyte origins of hES and pSC cells. IPS cells can be generated from adult somatic cells, which are reprogrammed by ectopic transduction of specific reprogramming factors (Fig. 1) (Takahashi et al. 2007). Induced PS cells are characterized by their unlimited self-renewal in vitro and their capability to differentiate into all somatic cell types (Takahashi et al. 2007). Induced PS cells can be derived from any tissue; this should allow an autologous patient-specific therapy without immunosuppression (Takahashi et al. 2007). Murine and human iPS cells seem to be indistinguishable from ES cells by morphology, stemness-related gene expression, epigenetic state, and differentiation potential (Daley et al. 2009). Murine iPS cells can contribute to chimera formation and germ line transmission, a test which is not possible in humans due to ethical concerns. The pluripotent features make iPS cells a promising tool for cell therapy approaches on an autologous basis, as well as for disease modeling, and drug screening in vitro (Kim et al. 2011; Ma et al. 2014; Spitalieri et al. 2016; Nishio et al. 2016).

Commonly, iPS cells are generated by retroviral or lentiviral gene transfer of different combinations of reprogramming genes, typically including OCT4, SOX2, KLF4, c-MYC, NANOG, and LIN28 (Takahashi and Yamanaka 2006; Okita et al. 2007; Takahashi et al. 2007; Yu et al. 2007). Recently, combinations with other reprogramming factors were found to be effective too (Buganim et al. 2014), emphasizing that improvements of the reprogramming processes with regard to the used reprogramming genes might be possible. However, the viral insertions of the reprogramming factors are associated with considerable risks for genome integrity. Alternative methods such as non-integrating adenoviral vectors (Stadtfeld et al. 2008), plasmids (Yu et al. 2009), recombinant proteins (Zhou et al. 2009), modified messenger RNAs (mRNAs) (Warren et al. 2010), and small epigenetic modifier molecules (Shi et al. 2008) were employed for iPS cells derivation (Talluri et al. 2015). However, the efficiency of reprogramming using these methods is significantly lower than that of retroviral or lentiviral vectors (Kumar et al. 2015a).

More recently, transposon-mediated reprogramming to iPS cells (Kumar et al. 2015b) has emerged as useful alternative to virus-mediated reprogramming of somatic cells from human origin (Davis et al. 2013; Inada et al. 2015), non-human primates (Debowski et al. 2015), and farm animal species, such as pig (Kues et al. 2013), horse (Nagy et al. 2011; Nagy and Nagy 2015), and cattle (Talluri et al. 2015). Transposons are non-viral mobile genetic elements that are capable of self-directed integration into the host genome (Urschitz and Moisyadi 2013). Several non-autonomous transposon systems catalyzing this cut and paste mobilization have

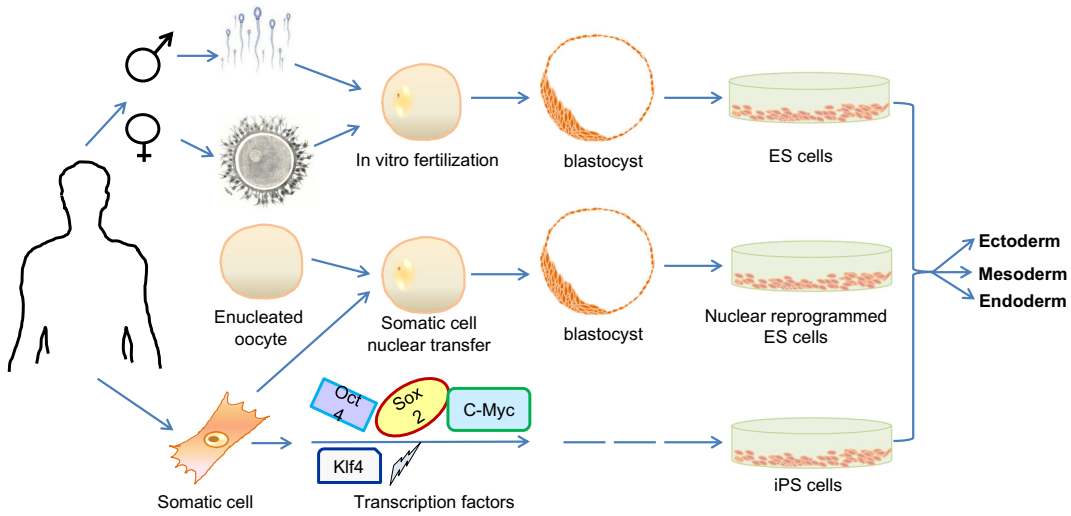

Fig. 1 Derivation of human pluripotent stem cells. Human ES are derived from early embryos, which are destroyed in this step. In contrast, iPS cells can be derived from adult somatic cells. Therefore, a patient's biopsy is used to grow primary somatic cells, which are transduced with core reprogramming factors (*OCT4*, *SOX2*, *KLF4*, *c-MYC*) to induce pluripotency. The iPS cells have an unlimited self-renewal and the capability to differentiate into all cell types. After sufficient amplification, the iPS cells are triggered to differentiate into the desired mature cell types

been described and among them engineered derivatives of piggyBac (PB) and Sleeping Beauty (SB) have been used recently for reprogramming approaches (Vargas et al. 2016). The respective transposases recognize and bind to specific inverted terminal repeat (ITR) flanked transposons, cut this DNA element from the donor, and reinsert it into the recipient genome (Urschitz and Moisyadi 2013; Kumar et al. 2015b). In contrast to the biased integration preference of most disarmed viruses used for gene transfer, SB transposon integrations appear to happen at random and without a bias for promoter and gene-containing regions (Gogol-Döring et al. 2016). The integrated transposon can be removed seamlessly in case of PB or with a minimal footprint in case of SB by resupplying the respective transposase, which makes the system more attractive and relevant in producing safe and clinically suitable iPS cells (Fraser et al. 1996; Woltjen et al. 2011). Here, we review the clinical potential of human iPS cells and discuss potential risks, which may be associated with iPS cell-based applications.

Potential risks/uncertainties of iPS cells and possible solutions

The potential risk factors for clinical application of iPS cells include the cell source, the in vitro culture, the reprogramming method, and the site of application (http://www.ich.org/products/guidelines.html; Fig. 2). Risk evaluation regarding the use of iPS cells includes the possible occurrence of teratoma formation from unintentionally transplanted pluripotent stem cells. Thus, any iPS-derived cell transplant should be free of residual undifferentiated and pluripotent cells (Ben-David and Benvenisty 2011; Cunningham et al. 2012). In human iPS-derived differentiated cells, residual pluripotent cells could be removed with antibodies against pluripotency surface markers, such as stage-specific embryonic antigen-5 (SSEA-5), CD9, CD30, CD50, CD90, and CD200 (Tang et al. 2011). In addition, chemical ablation of undifferentiated iPS cells is feasible, e.g., by targeting tumorigenicity-associated proteins (Lee et al. 2013; Cunningham et al. 2012; Ben-David et al. 2013; Ben-David and Benvenisty 2014; Sugai et al. 2016). Recently, a real-time monitoring system allowing the quantification of undifferentiated iPS via their electrochemical potential was presented (Yea et al. 2016); such direct and label-free systems will significantly contribute to the development of safe iPS-derived transplants.

In addition, the reprogramming method to induce cellular pluripotency may itself increase the potential risk of cancer induction. In vitro cell culture of the fibroblasts and the gene transfer of reprogramming factors could result in the accumulation of pre-existing mutations, the integration-induced mutagenesis, and

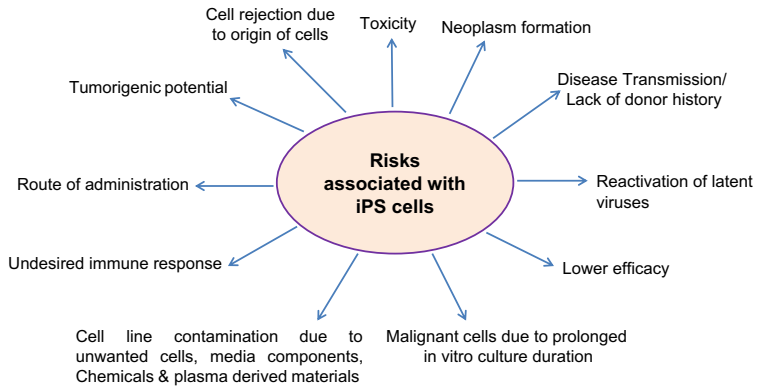

Fig. 2 Overview of potential risks associated with iPS cell-based therapies

resetting the epigenetic codes of the genome during reprogramming (Blasco et al. 2011; Wu and Dunbar 2011; Inoue et al. 2014).

Chemical and genetic strategies directed to lowering reprogramming-induced replication stress are currently tested to reduce genomic instability of iPS cells (Ruiz et al. 2015). Genetic instability is a specific problem in the reprogramming of cells from patients, which carry disease-causing genes with expansions of repeat regions, such as the X-linked fragile X mental retardation 1 (FMR1) (Haston and Finkbeiner 2016; Mor-Shaked and Eiges 2016). The repeat regions are per se instable, and reprogramming and in vitro differentiation can negatively affect on repeat instability and epigenetic regulation of the affected gene regions (Sareen et al. 2013; Grunseich et al. 2014). However, a comparative analysis of a collection of cell lines carrying different repeat variations provide a unique opportunity to assess which modification causes the pathological pathways in the patients (Mor-Shaked and Eiges 2016). Therefore, it is recommended to cryopreserve primary passages of cells for comparative analyses (Santostefano et al. 2014). Recent studies suggested that the use of high-resolution methods for continuous monitoring of genomic alterations throughout reprogramming could improve the quality of the iPS cells (Kang et al. 2015). The genomic instability of the iPS cells could also be reduced via selection of an appropriate starting cell source and optimized reprogramming and differentiation conditions (Yoshihara et al. 2016).

The possibility of a spontaneous reactivation of the reprogramming factors in differentiated cell derivatives has never been completely ruled out, and the risk of tumor generation after application of iPS cell-derived mature cells is still present. At least in in vitro differentiation assays, the spontaneous reactivation of lentivirally transduced reprogramming factors and secondary reprogramming events can be found (Galat et al. 2016). The rate of teratoma formation is also influenced by the site and mode of administration of the stem cells (Prokhorova et al. 2009). Transplanted murine ES cells caused malignant teratocarcinomas in an autologous setting, while xenotransplantation in rats did not (Erdo et al. 2003). Similar observations have been reported for human ES cells by Shih et al. (2007). An increased tumor growth was found after human ES cells were injected into human fetal tissue engrafted in severe combined immunodeficiency (SCID) mice, while differentiated teratomas formed after direct injection into SCID mouse tissue (Knoepfler 2009; Cebrian-Serrano et al. 2013).

Apart from genome alteration caused by viral insertion, genome integrity is still a concern. Gore et al. (2011) reported genomic mutations in iPS cells that were reprogrammed through diverse methods. To retain the genome integrity, various reprogramming methods employing episomal vectors, plasmids, recombinant proteins, modified mRNAs, Sendai virus vectors, and small epigenetic modifier molecules have been tested (Seki and Fukuda 2015; Kumar et al. 2015a). Brambrink et al. (2008) proposed doxycycline-regulated expression systems to reduce the risk of an uncontrolled reactivation of the reprogramming factors. Therefore, the generation of iPS cells through non-viral gene transfer is a promising alternative. This include the use of chromatin-modifying molecules, such as trichostatin A, valproic acid, or 5-aza-deoxycytidine, to induce pluripotency related genes in somatic cells (Ruau et al. 2008; Steliou et al. 2012) or to reduce or replace the reprogramming factors (Huangfu et al. 2008; Habib et al. 2013; Hermann et al. 2016). Recently, a modified clustered regularly interspaced short palindromic repeats, CRISPR-associated protein 9 (CRISPR/Cas9) system was successfully applied to

directly convert fibroblasts to neuronal cells by the up-regulation of endogenous transcription factors critically for neuronal lineage (Black et al. 2016), suggesting an alternative approach to the gene delivery of exogenous reprogramming factors. The improvement in culture conditions and application of exogenous stimulation with leukemia inhibitory factor, small molecule inhibition of ERK1/ERK2 and GSK3β signaling (Ishii 2014), achieved the maintenance of pluripotent stem cells in the naive ground state (Gafni et al. 2013; Theunissen et al. 2014).

In general, the number of cells required for a therapeutic effect, their rate of retention, and cell survival for obtaining maximal clinical benefit are not known. The injection of concentrated cells into tissue may have unwanted effects due to formation of aggregates (Prockop and Olson 2007).

In vitro conditions and cellular expansion itself can affect the genomic integrity of the cultured cells. Every cell division can result in DNA mutations (Herberts et al. 2011). Cell culture-induced copy number changes and loss of heterozygosity for both human iPS cells and ES cells have been reported (Narva et al. 2010; Laurent et al. 2011). In contrast, one report mentioned that genomic rearrangements in human iPS cells disappear after extended culture owing to selective disadvantages of the mutated cells (Hussein et al. 2011). During in vitro culture, chromosomal aberrations/alterations can occur (Werbowetski-Ogilvie et al. 2009). The chromosomal aberrations of human iPS cells can originate from the somatic cells before reprogramming and/or might be induced during the reprogramming process and extended culture.

The degree of chromosomal instability in canine iPS cells has been determined by Koh et al. (2013) through utilizing high-resolution comparative genomic hybridization assays. In human iPS cells, gained chromosomal aberrations and karyotypic abnormalities at high passages were found (Baker et al. 2007; Josephson 2007; Mayshar et al. 2010). The combined application of array comparative genomic hybridization and fluorescence in situ hybridization provides an analytical tool to evaluate cell lines before being used in regenerative medicine (Hussein et al. 2013). The analysis of 127 independent mouse iPS cell lines revealed a high incidence of trisomies of chromosomes 8 and 11 (Ben-David and Benvenisty 2012). The genetic fidelity of 22 human iPS cell lines generated by different reprogramming methods was investigated and a mean of five point mutations in protein-coding genes could be detected (Gore et al. 2011). Interestingly, half of these mutations were already present before reprogramming, while the others were accumulated after reprogramming (Mayshar et al. 2010; Gore et al. 2011; Taapken et al. 2011).

The altered expressions of the endogenous versions of the reprogramming factors have also been reported to cause diseases (Singh et al. 2015). For example, the deviant expression of Sox2 has been reported to cause mucinous colon carcinoma (Park et al. 2008). KLF4 plays an important role in the formation of breast tumors (Ghaleb et al. 2005; Singh et al. 2015). The ectopic expression OCT4 in somatic cells may promote tumorigenesis (Wang et al. 2013). Additionally, the expression of OCT4 protein has been detected in tumors of germ cell (Jones et al. 2004) and in somatic organs (Liu et al. 2011; Hatefi et al. 2012). c-MYC has a prominent role in the formation of around 70% of human cancers (Kuttler and Mai 2006; Singh et al. 2015). However, small molecules and other factors, such as Esrrb (Feng et al. 2009), Tbx3 (Han et al. 2010), Glis1 (Maekawa et al. 2011), and L-MYC (Li et al. 2016), can be used to replace c-MYC during reprogramming. The omission of c-MYC may decrease the reprogramming efficiency but does not alter subsequent differentiation (Habib et al. 2013). The decrease in reprogramming efficiency may be compensated by specifically targeting regulatory microRNA pathways (Deng et al. 2015)

Recently, Zhang et al. (2012) reported that out of several hundred upregulated genes in iPS cells, about one third was also found to be overexpressed in cancer tissues. Five oncogenes were found to be overexpressed in iPS cells, and the oncogene RAB25 was found to be expressed exclusively in cells derived from iPS cells (Zhang et al. 2012; Singh et al. 2015). However, the safety of PSC-derived products needs to be fully addressed and must be demonstrated by ample preclinical data (Simonson et al. 2015). These data should include assays for chromosomal stability and for mutations in oncogenes and housekeeping genes; however, no standards for testing genetic mutations in therapeutic cell lines have been developed yet (Ma et al. 2014; Simonson et al. 2015; Trounson and DeWitt 2016). High throughput whole genome sequencing of iPS and iPS-derived cells would be one possibility to address this topic.

Large animal models for assessing and minimizing the risks of innovative cell therapies

The discovery of iPS cells allows a better understanding of pluripotency and disease development and inspires to

developing innovative cell therapies (Wang and Na 2011; Wang et al. 2015; Spitalieri et al. 2016; Barral and Kurian 2016). To ensure clinical safety and obtain reliable therapeutic results, standardized and normalized processes must be followed during human iPS cell-based therapies; the cell handling processes should be conducted under good manufacturing practice (GMP)-controlled environments and with reagents free of xenogeneic molecules (Jonlin 2014; Wang et al. 2015). Traditionally, rodents are used as animal models, because knockout or knock-in gene mutants are frequently available in these species (Trounson and DeWitt 2016). Immunoincompetent mice tolerate human grafts, and humanized mice provide models, which more closely resemble transplants performed in immune-competent humans (Schroeder and DiPersio 2011; Trounson and DeWitt 2016). However, cell therapies in rodent models do not always allow to predict the response in genetically heterogenous human diseases (Kastner and Gauthier 2008; Kehinde 2013). Limitations of murine models for pre-clinical assessments of potential cell therapies are the short life span, the small size, and the high level of inbreeding in this species (Kues et al. 2013). These shortcuts can be overcome by non-human primates (NHPs), which represent an extremely valuable potential biomedical model because of their close phylogenetic relationship to humans (Cebrian-Serrano et al. 2013). Induced PS cells have recently been derived from transgenic Huntington's disease (HD) monkeys and differentiated in vitro into neuronal cell types with typical HD-like features (Chan et al. 2010). Such studies may yield attractive in vitro and in vivo platforms for investigating HD pathogenesis and therapy. However, the use of NHPs in research is ethically questionable, expensive, and is not widely available (Cebrian-Serrano et al. 2013).

Large farm animals seem to be suitable to assess obstacles and risks in longitudinal pre-clinical tests of novel cell therapies, as well as of enhanced pharmaceutical and regenerative studies (Wolf et al. 2014; Kumar et al. 2015a; Flisikowska et al. 2016; Watson et al. 2016). In contrast to rodent models, they are more similar to humans with respect to life span, physiology, metabolism, and pathophysiology (Plews et al. 2012; Fan and Lai 2013; Kurome et al. 2013; Bassols et al. 2014). Large mammalian models will also allow in determining required cell doses to obtain therapeutic effects to follow the fate of transplanted cells and their functional integration in the host tissue (Duranthon et al. 2012; Wolf et al. 2014, Kumar et al. 2015a). In addition, the use of naturally occurring diseases in domestic animals, such as cancer or other chronic diseases, offers an invaluable comparative model for research (Cebrian-Serrano et al. 2013). Among domestic animals, the pig is an attractive model for preclinical testing of safety and efficacy of cell based therapies (Fig. 3; Fan and Lai 2013; Dolezalova et al. 2014; Gün and Kues 2014). The similarity in anatomy, size, and physiology of porcine organs to human organs pre-determine the pig as a suitable model for cardiovascular diseases, cancer, diabetes, ophthalmological diseases, neuronal disorders, and xenotransplantation (Holm et al. 2016; Wolf et al. 2014; Schook et al. 2016; Yao et al. 2016). The very recent development of efficient genetic engineering tools, such as zinc finger nucleases, TALEN, CRISPR/Cas9, Sleeping Beauty, and piggyBac transposons, and the direct translation for precision genetic modifications in large animals allow the targeted design of genetically modified pigs for specific disease phenotypes (Garrels et al. 2012; Garrels et al. 2016; Bosch et al. 2015; Watson et al. 2016; Schook et al. 2016; Yao et al. 2016).

Most animal iPS cell lines from non-rodent species were not assessed for chimera contribution so far (Kumar et al. 2015a). Preliminary studies suggest that porcine iPS cells can contribute to chimera formation; however, so far, only low frequencies of chimera formation were achieved (West et al. 2010; Fujishiro et al. 2013). The pig has also been used as a model for retinal stem cell transplantation, where porcine iPS cells differentiated in vitro into the rod photoreceptor lineage and, after transplantation, engrafted cells integrated into the outer layer of the retina (Zhou et al. 2011; Cebrian-Serrano et al. 2013). Templin et al. (2012) used a novel long-term cell imaging approach in a pig model by transplanting human cells, which carried a sodium iodide symporter. Importantly, no signs of human cell-derived tumor formation were detected. In another study, porcine iPS cells were differentiated into endothelial cells and transplanted into mice of a myocardial infarction model (Gu et al. 2012). Recently, human iPS cell-derived cardiovascular cells have been evaluated in a porcine model for acute myocardial infarction; the results showed improvements in myocardial wall stress, metabolism, and contractile performance, and importantly, no signs of ventricular arrhythmias (Ye et al. 2014) were observed.

Apart from these, porcine iPS cells have also successfully been used for in vitro differentiation into

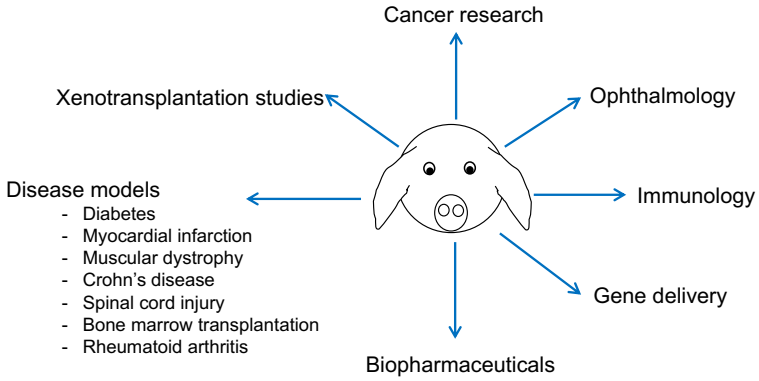

Fig. 3 Most suitable animal model for assessing safety aspects of iPS cells. Since physiology, anatomy, pathology, genome organization, body weight, and life span of the domesticated pig are similar to those of humans, the *pig* represents an excellent biomedical model for translational research

cardiomyocyte-like (Montserrat et al. 2011), neuron-like (Kues et al. 2013; Gallegos-Cárdenas et al. 2015), astrocyte-like, oligodendrocyte-like, and hepatocyte-like cells (Aravalli et al. 2012). It also exhibits the potential to provide useful targets in which testing iPS cell efficacy to treat specific diseases, such as Alzheimer's disease, HD, retinitis pigmentosa, spinal muscular atrophy, and diabetes, can be carried out (Cebrian-Serrano et al. 2013).

Advancements in clinical applications of iPS cells

One of the greatest promises of the induction of cellular pluripotency is the generation of patient-specific cell replacement therapies (Robinton and Daley 2012). The development of the iPS cell technology has brought this aim closer to reality. However, concerns regarding the use of viral vectors and reactivation of the potent oncogenes c-MYC and KLF4 need to be addressed. Integration of these genes into the host genome can result in oncogenesis (Li et al. 2002; Hacein-Bey-Abina et al. 2003; Howe et al. 2008). Furthermore, for clinical application, iPS cells should have the ability to develop into terminally differentiated cell types, which can integrate as functional derivatives in situ (Dimmeler et al. 2014; Trounson and DeWitt 2016).

An important reason of using derivatives of autologous iPS cells for transplantation is avoiding immune rejection. Recently, we had applied a novel method for differentiation of mouse iPS cells to lentoid bodies expressing a lens cell-specific fluorescent reporter (Anand et al. 2016). We speculate that the obtained knowledge can be translated to optimize lens cell differentiation of human iPS cells and thus to advance the growth of patient-specific lentoid bodies. Probably the first application of iPS cells will be the treatment of eye disease. In this regard, the phase 1 clinical trial of autologous human iPS cell-derived retinal pigmented epithelium cell for the treatment of age-related macular degeneration was started at the RIKEN Centre for Developmental Biology, Japan, in 2014. One patient has been treated with retinal pigment epithelium (RPE) cells derived from autologous iPS cells (http://www.dddmag.com/articles/2014/10/japan-starts-world-first-stem-cell-trial-plans-more). But, the second transplantation was canceled due to the observation of genetic mutations in the candidate's iPS cell-derived cells (Garber 2015). Then, the investigators revised their protocol from an autologous to an allogeneic source of the RPE cells to increase the safety and reduce the risk of tumorigenicity (Gouda and Takeishi 2015). Recently, it has been identified that the key regulatory genes involved in limbal development, differentiation, and expansion are likely to accelerate the therapeutic opportunity of human iPS cells (Pellegrini and De Luca 2014; Trounson and McDonald 2015). In another study, Carpenter et al. (2012) injected human iPS cells-derived cardiac progenitor cells into the pre-infarct hearts of rats and found that these cells were able to differentiate into cardiomyocytes and smooth muscle fibers and were retained in the rat hearts for at least 10 weeks after myocardial infarction (Carpenter et al. 2012). Earlier experiment has demonstrated the proof of principle that human iPS cells derived from ALS patients could be differentiated into motor neurons (Dimos et al. 2008). Trials of iPS cells based therapies for Parkinson disease are also under consideration (science-health/kyoto-universitys-potential-ips-celltherapy-parkinsons-may-delayed/#.VkUsbunoD8F). Thus, therapeutic applications of iPS cells are rapidly expanding. These stem cells can be efficiently utilized in regenerative medicine, tissue engineering, disease modeling, drug development and

discovery, and genetic therapies (Fig. 4; Nelson et al. 2010; Lengerke and Daley 2010; Nordin et al. 2011; Kimbrel and Lanza 2015; Jiang et al. 2012; Teoh and Cheong 2012). In this regard, researchers are now generating disease specific iPS cells from somatic cells of patients with a variety of diseases, including ALS, adenosine deaminase deficiency-related severe combined immunodeficiency, Gaucher disease type III, Duchenne and Becker muscular dystrophies, Parkinson's disease, HD, type 1 diabetes mellitus, and spinal muscular atrophy (Dimos et al. 2008; Park et al. 2008; Ebert et al. 2009; Al-Anazi 2015; Trounson and DeWitt 2016). These iPS cells generated from patients diagnosed with a specific genetically inherited disease could then be useful as model for a therapeutic intervention.

A recent concept is to develop homozygous HLA-typed iPS cell banks that could be feasible for achieving generalized therapies (Nakatsuji et al. 2008; Okita et al. 2011; Taylor et al. 2012), instead of iPS cell-derived transplants from allogeneic donors with foreign HLA, which requires lifelong immunosuppressive treatment of the recipients (Seki and Fukuda 2015). This type of approach is already progressing (Cyranoski 2012; Seki and Fukuda 2015). However, rigorous testing and abundant sources of these cell types are needed for pre-clinical research to generate data for regulatory approval for human studies (Trounson and DeWitt 2016). The cells also need to be manufactured in large quantities with GMP for clinical trials (Baghbaderani et al. 2015; Trounson and DeWitt 2016). Wang et al. (2015) generated integration free clinical-grade human iPS cell lines under GMP controlled conditions. The scalable expansion of human iPS cells in suspension bioreactors has the potential to overcome limitations of adherent culture with respect of yield and purity (Kempf et al. 2015; Kropp et al. 2016). Further comprehensive discussions of genetic, genomic, logistic, and ethical challenges for the clinical translation of human iPS cell-derived transplants were highlighted in a number of recent reviews (Neofytou et al. 2015; Tapia and Schöler 2016; Seki and Fukuda 2016).

Challenges and perspectives

Currently, only few clinical trials are in progress for iPS cell derivatives. The establishment of iPS cells has imposed huge impact on new concepts to exploit the modulation and direction of cellular potency for

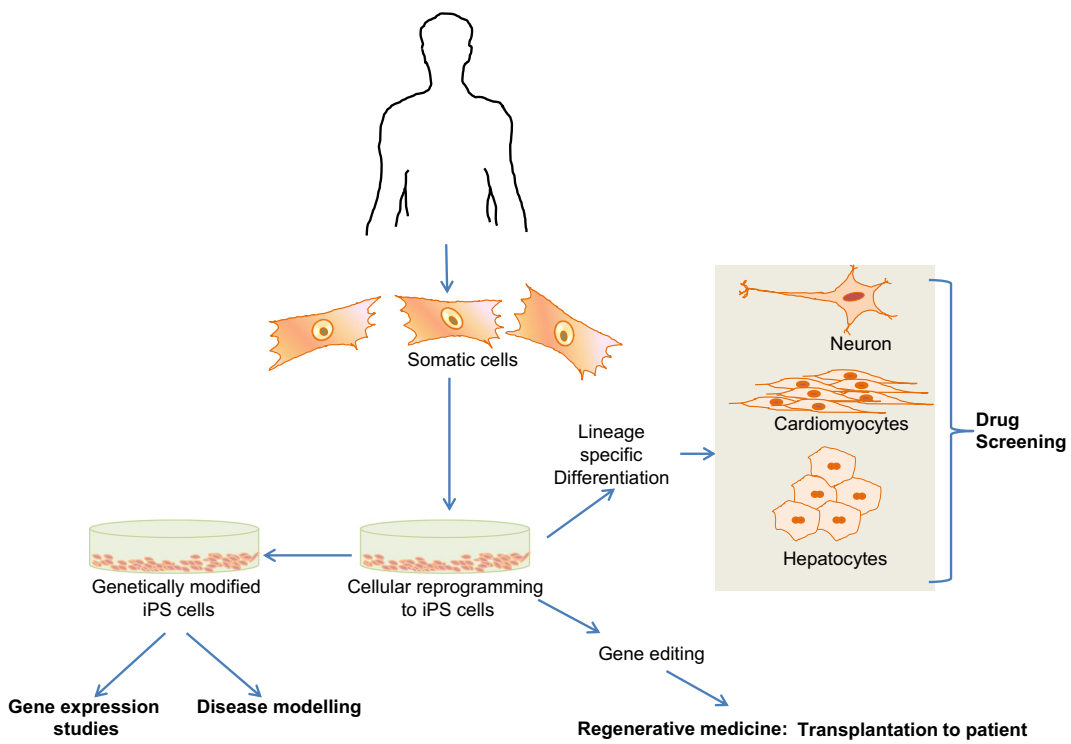

Fig. 4 Applications of human iPS cells. Potential of human iPS cells for drug screening, disease modeling, and therapeutic approaches

therapeutic approaches. However, potential iPS cell therapies have inherent concerns and challenges, which are currently limiting the translation to clinical applications, including the efficient and consistent productions of iPS cells, biased differentiation of iPS cells, heterogeneity of cells derived from iPS cells, and lack of routine direct and label-free characterization of human iPS cells and their cellular derivatives (Nordin et al. 2011; Al-Anazi 2015). Specific, sensitive, and high-throughput analytic tools for informative genetic and epigenetic characterization are not widely used at the moment but are imperative for the translation into clinical approaches. In addition, to overcome the current barriers and limitations, it will be essential to define minimal consensus criteria for the evaluation of iPS cells, to harmonize regulatory standards, to agree on robust and sensitive methods for the functionality of iPS-derived cells, and to design surveillance programs of patients in preliminary clinical studies to turn these novel cell applications into safe therapies (Azuma and Yamanaka 2016).

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