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Induced Pluripotent Stem Cells and Induced Pluripotent Cancer Cells in Cancer Disease Modeling

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Abstract

In 2006, Noble Prize laureate Shinya Yamanaka discovered that a set of transcription factors can reprogram terminally differentiated somatic cells to a pluripotent stem cell state. Since then, induced pluripotent stem cells (iPSCs) have come into the public spotlight. Amidst a growing field of promising clinical uses of iPSCs in recent years, cancer disease modeling has emerged as a particularly promising and rapidly translatable application of iPSCs. Technological advances in genome editing over the past few years have facilitated increasingly rapid progress in generation of iPSCs with clearly defined genetic backgrounds to complement existing patient-derived models. Improved protocols for differentiation of iPSCs, engineered iPSCs and embryonic stem cells (ESCs) now permit the study of disease biology in the majority of somatic cell types. Here, we highlight current efforts to create patient-derived iPSC disease models to study various cancer types. We review the advantages and current challenges of using iPSCs in cancer disease modeling.

Keywords

Cancer disease model · Genome editing · Induced pluripotent cancer cells · Induced pluripotent stem cells · Reprogramming

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Abbreviations

AML	acute myeloid leukemia
APC	adenomatous polyposis cell
CML	chronic myeloid leukemia

COs	colorectal organoids
ER	estrogen receptor
ESCs	embryonic stem cells
FAP	familial adenomatous polyposis
HBOC	hereditary breast and ovarian cancer
iPCCs	induced pluripotent cancer cells
iPSCs	induced pluripotent stem cells
JMML	juvenile myelomonocytic leukemia
LFS	Li-Fraumeni syndrome
LSC	leukemic stem cells
MDS	Myelodysplastic syndrome
MSCs	mesenchymal stem cells
NS	Noonan syndrome
PDAC	pancreatic ductal adenocarcinoma
PR	progesterone receptor
sgRNA	single guide RNA
TALEN	transcription activator-like effector nuclease
ZFN	zinc finger nuclease

1 Introduction

In 2006, Kazutoshi Takahashi and Shinya Yamanaka pioneered the induction of pluripotent stem cells, termed induced pluripotent stem cells (iPSCs), from mouse embryonic or adult fibroblasts by inducing expression of four transcription factors, Oct4, Sox2, Klf4, c-Myc (referred to as the "four Yamanaka factors"), and growing the cells under mouse embryonic stem cell (ESC) culture conditions (Takahashi and Yamanaka 2006). Later, Shinya Yamanaka's and James A. Thomson's research groups successfully demonstrated the reprogramming of adult human fibroblasts as well as differentiated adult human somatic cells to human iPSCs (Takahashi et al. 2007; Yu et al. 2007). These iPSCs demonstrated gene expression, morphology, pluripotency gene epigenetic profiles and three germ-layer differentiation capacity that was comparable to ESCs. The technique of reprogramming differentiated adult cells back to pluripotent iPSCs has paved the way for the creation of patient-specific iPSC lines that has revitalized the field of both stem cell research as well as personalized medicine.

Soon after the first reports of iPSC creation by transcription factors, many groups confirmed these findings both in mice (Maherali et al. 2007; Wernig et al. 2007) and humans (Park et al. 2008b; Lowry et al. 2008). Early progress was limited by the low efficiency of iPSC generation, typically less than 0.1% of transfected fibroblasts (Takahashi and Yamanaka 2006; Takahashi et al. 2007; Yu et al. 2007; Park et al. 2008b). Initially, iPSCs were generated using either retroviruses or lentiviruses. Mouse iPSCs derived retrovirally are apparently normal, but retroviruses may cause iPSCs to be immunogenic (Zhao et al. 2011; Nakagawa et al. 2008; Aoi et al. 2008), limiting their application in animal models. Lentiviruses and some retroviruses can infect both nondividing and proliferating cells, limiting selectivity of the reprogramming process. Finally, because retroviruses or lentiviruses induce genomic integration of the targeted genetic material, it is impossible to fully guard against insertional mutagenesis. Thus, to reduce the risks associated with translational applications of iPSCs, many integration-free methods for iPSCs generation have been reported. These methods include adenovirus (Stadtfeld et al. 2008; Zhou and Freed 2009), Sendai virus (Fusaki et al. 2009; Seki et al. 2010; Ban et al. 2011), mRNA transfection (Warren et al. 2010), miRNA infection/transfection (Subramanyam et al. 2011; Anokye-Danso et al. 2011), Piggy Bac (Kaji et al. 2009; Woltjen et al. 2009; Mali et al. 2010), minicircle vectors (Narsinh et al. 2011), episomal plasmids (Okita et al. 2008; Hu et al. 2011), and direct protein insertion (Zhou et al. 2009; Kim et al. 2009). Among these, episomal plasmids and Sendai viruses are now the most commonly used tools for iPSC research.

The maturation of genome editing technologies over recent years has now facilitated making arbitrary genetic modifications to iPSCs, for example introducing a particular oncogenic mutation into patient-derived wild-type iPSCs or correcting a mutation in patient-derived mutant iPSCs (Hockemeyer and Jaenisch 2016). While numerous genome editing systems exist, CRISPR/Cas9 technology has been proven to be particularly useful in stem cell research and

human disease modeling (Cong et al. 2013; Matano et al. 2015; Schwank et al. 2013), as it affords that DNA-binding specificity that is encoded solely by the single guide RNA (sgRNA). Zinc finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) platforms are also used to engineer hPSCs (Sexton et al. 2014; Soldner et al. 2011), though these approaches are often more costly and labor-intensive and less efficient than CRISPR/Cas9.

2 iPSCs in Cancer Disease Modeling

While the generation of iPSC lines (whether from affect patient fibroblasts or healthy donors) with unique genetic backgrounds represents an impressive scientific feat on its own, the full potential of this technology is only realized in conjunction with well-defined differentiation protocols. With appropriate such protocols, the effect of the discrete genetic alteration can be serially interrogated on a specific differentiated cell type and all of its progenitors, opening avenues for "disease modeling in a dish".

Many research groups have implemented iPSC modeling to better understand the underlying molecular mechanisms governing human diseases as well as to better study targeted therapies. iPSC lines have been produced to model various human diseases, including Huntington's Disease (An et al. 2012), Alzheimer's Disease(Israel et al. 2012; Doulatov et al. 2017; Kondo et al. 2013), Parkinson's disease (Kriks et al. 2011; Devine et al. 2011; Nguyen et al. 2011), Down syndrome (Briggs et al. 2013), familial dysautomania (Lee et al. 2009), cardiomyopathy (Carvajal-Vergara et al. 2010; Ang et al. 2016; Yazawa et al. 2011; Moretti et al. 2010; Itzhaki et al. 2011; Karakikes et al. 2014), liver metabolic disorders (Rashid et al. 2010; Yi et al. 2012), amyotrophic lateral sclerosis (Richard and Maragakis 2015), and urinary and prostate tract diseases (Moad et al. 2013).

One additional natural application of iPSCs that has only recently come to attention is cancer modeling. Because cancer is fundamentally a genetic disease, a select number of researchers have therefore begun to apply iPSC and reprogramming methods as well as induced pluripotent cancer cell (iPCC) technology to better understand the process of oncogenesis and offer novel treatment approaches (Fig. 1).

2.1 Li-Fraumeni Syndrome

Li-Fraumeni syndrome (LFS) is a genetically inherited autosomal dominant familial syndrome due to germline p53 mutations and characterized by a high incidence at a young age of a number of otherwise rare tumor types, including osteosarcoma, soft tissue sarcoma, breast cancer, gliomas, adrenocortical carcinoma and leukemia (Li and Fraumeni 1969; Zhou et al. 2017). Lee et al. generated LFS patient-derived iPSCs and explored the effect of the p53 mutation on osteoblastic lineages in order construct a disease modeling platform to explore the pathological mechanisms of mutant p53 in osteosarcomagenesis. Osteoblasts differentiated from LFS iPSC-derived mesenchymal stem cells (MSCs) recapitulated primary osteosarcoma-associated gene signatures and demonstrated impaired osteogenic differentiation ability (Lee et al. 2015). LFS iPSC-derived osteoblasts allow for the investigation of the role of mutant p53 in early osteosarcomagenesis prior to the acquisition of additional genomic mutations that are commonly observed in patient tumor samples. As p53 had been known to suppress H19 expression (Okita et al. 2008), Lee et al. investigated the influence of a p53 gain-of-function mutant on the expression of H19 in LFS iPSC-derived osteoblasts using transcriptomic analyses. The study indeed confirmed H19 downregulation in the p53 mutant and also demonstrated that H19 downregulation in part mediates the development of mutant p53-driven osteosarcoma.

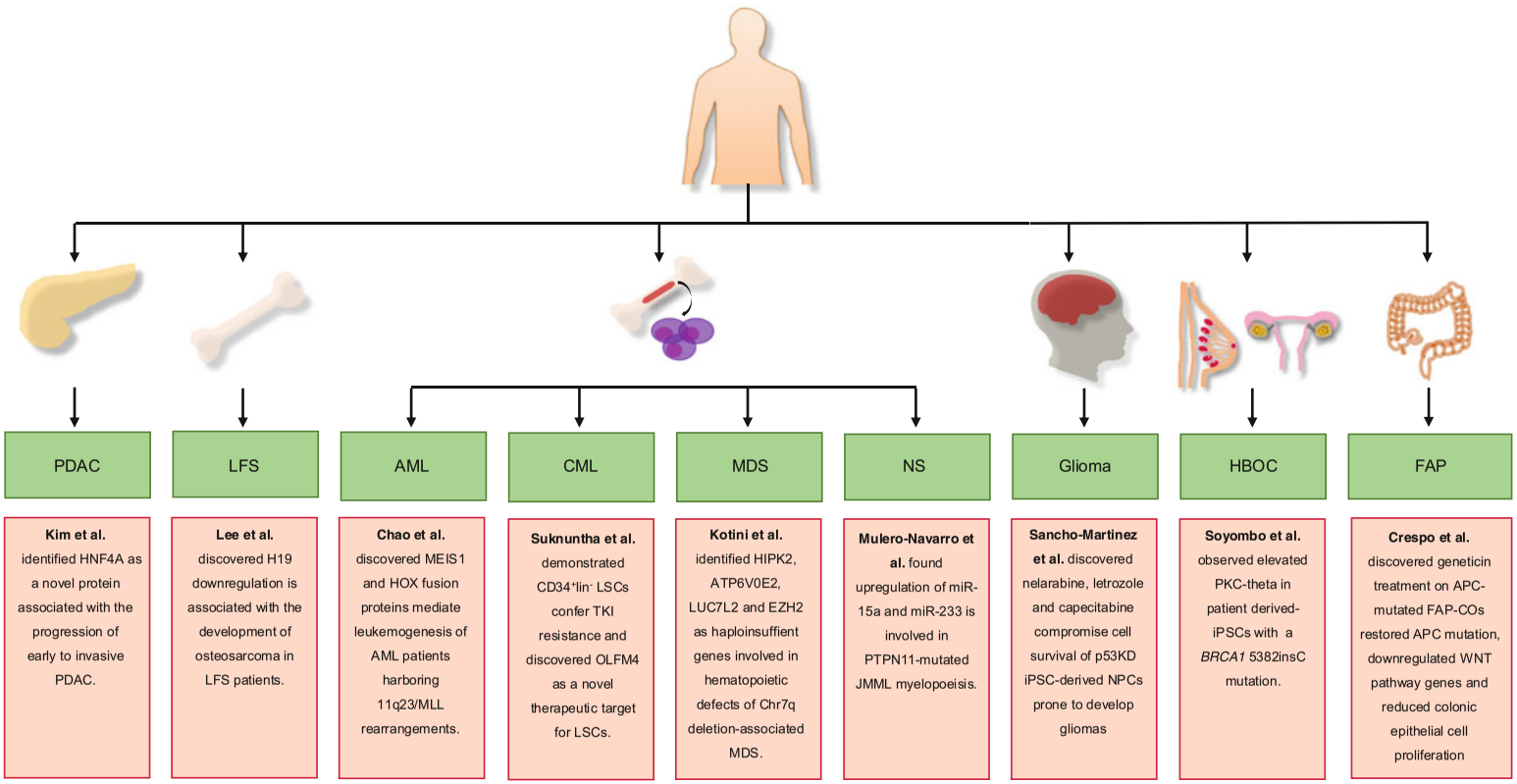

Fig. 1 Patient-derived iPSCs and iPCCs in cancer research. Taking advantage of their capability to differentiate into diverse cell types of the three germ layers, both iPSCs and iPCCs have been applied to model PDAC, LFS, AML, CML, MDS, NS, glioma, HBOC, and FAP

2.2 Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is a disease arising from transformation of hematopoietic cells harboring multiple genetic and epigenetic mutations as well as chromosomal rearrangements (Zwaan et al. 2015). Chao et al. established human AML iPSC lines carrying 11q23/MLL rearrangements by transducing myeloblasts with pluripotency reprogramming factors (OSKM) (Chao et al. 2017). These AML iPSC lines, when maintained in iPSC culture conditions, have reduced leukemic potential but reacquire their leukemic ability as well as genetic and epigenetic MLL signature expression patterns upon hematopoietic cell differentiation. Their findings show that the leukemogenesis can be driven by the reactivation of myeloid-specific MLL target genes within a background of expression of MEIS1 and HOX fusion proteins.

2.3 Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a disorder associated with the expansion and accumulation of myeloid progenitors in the peripheral blood and bone marrow (Rowley 1973). Expression of BCR-ABL in $CD34^+$ cells of CML patients has been linked to the pathogenesis of CML and BCR-ABL tyrosine kinase inhibitors (TKI) are now prescribed as first line treatment of CML (Druker et al. 2006). However, patients still have residual molecular evidence of CML post-treatment and leukemic stem cells (LSC) are thought to represent the reservoir of cells that permits persistence of CML post-treatment (Corbin et al. 2011). Suknuntha et al. established CML iPSCs from mononuclear cells of affected patients and subsequently generated LSC-like cells from differentiated CML iPSCs (Suknuntha et al. 2015). These LSC-like cells harbored primitive hematopoietic cell markers ($CD34^+$) but were negative for hematopoietic lineage markers (lin^-). Using the iPSC disease modeling platform, Suknunta et al. demonstrated resistance of this population to TKIs but was able to uncover olfactomedin 4 (OLFM4) as a novel agent with the potential to target the survival and proliferation of $CD34^+lin^-$ LSC-like cells.

2.4 Myelodysplastic Syndrome

Myelodysplastic syndrome (MDS) is a disease resulting from genetic mutations in hematopoietic stem cells. Some MDS patients can live with the disease for many years even with minimal clinical treatment, though others progress to develop AML (Sperling et al. 2017). However, the cellular mechanism by which MDS progresses to AML is not well understood. Kotiniet al. established patient-derived iPSCs that were able to recapitulate the entire progression spectrum of disease stages from MDS to transplantable leukemia (Kotini et al. 2015). Introducing a chr7q deletion into normal patient-derived iPSCs allowed for the modeling of pre-leukemia as well as transformed MDS. Using phenotype-rescue screening, they identified several distinct haploinsufficient genes (HIPK2, ATP6V0E2, LUC7L2 and EZH2) involved in producing the hematopoietic defects of chr7q deletion-associated MDS.

2.5 Noonan Syndrome

Noonan syndrome (NS) is an autosomal dominant disorder characterized by short stature, hypertelorism, webbed neck and exophthalmos (Noonan 1968; Roberts et al. 2013). Some patients with NS are also predisposed to developing malignant tumors including juvenile myelomonocytic leukemia (JMML). As both NS and JMML have been associated with gain-of-function PTPN11 mutations (Oishi et al. 2009), Mulero-Navarro et al. used hematopoietic cells differentiated from NS/JMML patient-derived iPSCs harboring PTPN11 mutations to investigate the role of PTPN11 mutations in NS-associated JMML (Mulero-Navarro et al. 2015). Hematopoeitic cells derived from NS/JMML patient iPSCs recapitulated aspects of the disease phenotype, including sensitivity to granulocyte-macrophage colony stimulating

factor as well as hyperproliferation of the myeloid population. Transcriptomic analysis comparing NS/JMML-derived $CD33^+$ myeloid cells with $C33^+$ control myeloid cells revealed that increased ERK activation and increased STAT5, an important component of JAK/STAT signaling pathway, was associated with development of JMML in NS patients. NS/JMML iPSC-derived $CD33^+$ myeloid cells also demonstrated increased proliferation and elevated expression of both miR-233 and miR-15a. By investigating the role of miRNAs in JMML pathogenesis, Mulero-Navarro et al. showed that upregulation of miR-233 alone is sufficient to induce PTPN11-mutated JMML myelopoiesis and that normal myelopoiesis can be restored through miRNA inhibition, a finding enabling novel therapeutic target for patients with JMML harboring PTPN11 mutations.

2.6 Pancreatic Ductal Adenocarcinoma

Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer-related death in the United States, with patient 5-year survival rates of less than 5% due to typically late-stage clinical presentation, local invasiveness adjacent to essential vasculature and biliary structures and the metastatic nature of the disease (Ying et al. 2016). Kim et al. reported successful reprogramming of one patient's late-stage PDAC cells harboring a typical KRAS mutation to iPSC-like cells (Kim et al. 2013). These PDAC iPSCs led to progression of invasive PDAC when transplanted into immunodeficient mice. Through proteomic analyses of the proteins secreted during progression of PDAC, Kim et al. were able to identify HNF4A as a novel protein associated with the progression of early to invasive PDAC.

2.7 Gliomas

Gliomas are one of the leading causes of CNS tumor-related deaths, with no current curative therapy available (Chen et al. 2012). Funato et al. discovered that neural progenitor cells (NPCs) could transform into glioma tumor-initiating cells (GTICs), leading to glioma development (Funato et al. 2014). As mutations affecting the p53 signaling pathway have been previously implicated in adult gliomas (Brennan et al. 2013), Sancho-Martinez et al. depleted p53 in wild-type iPSCs (so-called p53 "knockdown" or KD) and differentiated p53KD iPSCs to NPCs to investigate the mechanisms of gliomagenesis (Sancho-Martinez et al. 2016). These p53KD-NPCs were further transduced with mutant-active versions of SRC, EGFR and RAS to model activation of PI3K and MAPK pathways in adult gliomas (Guha et al. 2017). These genetically manipulated NPCs recapitulated GTIC properties *in vitro* and formed highly aggressive glioma-like tumors with the histopathological microstructure of clinical gliomas, namely undifferentiated stem cells and their differentiated derivatives. Sancho-Martinez applied this glioma iPSC disease modeling platform to discover three different chemical inhibitors (nelarabine, letrozole and capecitabine) whose exposure to GTIC-like cells compromised cell survival, highlighting the potential of this approach to generate potential glioma therapies.

2.8 Hereditary Breast and Ovarian Cancer Syndrome

Autosomal-dominant *BRCA1/2* mutations are the leading cause of hereditary breast and ovarian cancer (HBOC) syndromes (Futreal et al. 1994). Patients with inherited *BRCA1* mutations develop more aggressive breast cancers and at a younger age compared with patients with *BRCA2* mutations or sporadic breast cancers. The aggressiveness of tumors with *BRCA1* mutations could be due to *BRCA1*-deficient tumors commonly being estrogen receptor (ER) and progesterone receptor (PR) negative, suggesting that the tumors are driven by other oncogenes and precluding treatment with hormonal therapies (Turner et al. 2004). Soyombo et al. generated 24 iPSC lines (13 *BRCA1*-iPSCs and 11 wild-type iPSCs) from fibroblasts of patients carrying the Ashkenzaki

BRCA1 5382insC mutation to investigate the phenotype of patients with *BRCA1* mutation-associated tumors (Soyombo et al. 2013). All 24 iPSC lines showed embryonic stem cell-like morphology, expressed pluripotency markers and differentiation ability to all three germ layers. When comparing transcriptional profiles between *BRCA1* and wild-type iPSCs, Soyombo et al. discovered upregulation of *PRKCQ* expression, a gene that encodes for protein kinase C-theta (PKC-θ), in all 13 *BRCA1* iPSCs. They also detected elevated PKC-θ expression in more than half of primary tumor samples. As previous reports have linked PKC-θ activity to a subset of breast cancers (Gordge et al. 1996), results from Soyombo et al. support the potential of therapeutically targeting PKC-θ in patients with mutant BRCA1-associated cancers and possibly many other breast cancers.

2.9 Familial Adenomatous Polyposis

Familial adenomatous polyposis (FAP) is a rare familial cancer syndrome characterized by multiple colonic polyps and a very strong predisposition to colorectal cancer (Aaltonen et al. 1993). Germ line mutations discovered in the adenomatous polyposis cell (*APC*) gene have been linked to the pathogenesis of FAP (Nagase et al. 1992). To further investigate genetic roles in the pathogenesis of colorectal cancer, Crespo et al. generated colorectal organoids (COs) from FAP patient-derived iPSCs and discovered upregulation of WNT pathway genes in FAP-COs (Crespo et al. 2017). They found enhanced proliferation abilities of colonic epithelial cells within FAP iPSC-derived COs, consistent with the early-onset FAP patient phenotype. Crespo et al. also attempted to use the CO system as a disease modeling and drug screening platform. After screening XYZ drugs, they found that treatment of FAP-COs with the aminoglycoside antibiotic G418 (Geneticin) restored colonic epithelial cell proliferation to normal and downregulated WNT pathway-associated gene expression. These findings validate the concept of applying organoids for iPSC-based cancer or pre-cancer drug screening.

3 Advantages of iPSC Over Other Patient-Derived Cancer Models

Starting with the seminal studies by George Daley's group (Park et al. 2008a), a growing number of scientists have employed iPSCs for disease modeling. Patient-derived iPSCs retain several advantages compared with other competing systems for use in disease modeling and drug screening. First, iPSC-derived cells are suitable for high throughput drug screening to predict toxicity/therapeutic responses. Previous widely used models of drug screening include immortalized cell lines, tumor-derived cell lines, and patient tumor samples, but the availability and capacity of expansion are limited by difficulty in acquiring certain samples, senescence and/or low-fidelity cellular replication. In contrast, iPSCs can be passaged and expanded indefinitely without evidence of genomic alterations prior to differentiation towards a lineage of interest. Second, ethical issues are eliminated by use of patient iPSCs rather than ESCs. Since iPSCs are derived directly from the somatic tissues of patients, no human embryonic tissue or oocytes are ever created or destroyed (Yamanaka 2010; Nsair and MacLellan 2011; Yoshida and Yamanaka 2010; Stadtfeld and Hochedlinger 2010). Third, preclinical testing on human cells bypasses the common predicament of identifying therapies with high efficacy in a non-human animal system and no efficacy in humans. Expensive preclinical testing on animals for drug toxicity can also be somewhat reduced, for example by using iPSCs in various cytotoxicity assays. Patient-derived iPSCs offer the greatest fidelity possible to their ultimate target, the patient. Fourth, current gene editing technologies (including TALEN, CRISPR/Cas9 and ZNF) are very well-adapted to iPSCs and clear protocols have already been established. Generation and/or correction of disease-associated mutations in other cell types may require more time-consuming experimentation and optimization. Lastly, iPSC models leave the door open for future cell-based therapies. The lower immunogenicity of modified patient-derived iPSCs compared with existing

iPSC or ESC lines offers at least theoretical benefits if those cells are ever to be reintroduced into patients. Mouse studies have found no evidence of increased T cell proliferation or an antigen-specific secondary immune response after transplantation of mouse iPSC-derived embryoid bodies or tissue-specific cells (Guha et al. 2017).

4 Challenges

Despite the advancements in the application of iPSCs for cancer disease modeling (Gingold et al. 2016; Lin et al. 2017; Zhou et al. 2017; Papapetrou 2016), obstacles surrounding this platform still exist. One of the main challenges of the iPSC cancer modeling system is the technical reprogramming of cancer predisposition syndrome-associated somatic cells to iPSCs. Genetic alterations associated with cancer-associated genes may affect the efficiency of iPSCs reprogramming, preventing or inhibiting the induction of pluripotency. For example, genetic mutations associated with Fanconi anemia have been shown to resist pluripotency induction, resulting in inefficient iPSC reprogramming (Raya et al. 2009). Also, reprogramming cancer cells to iPSCs, also known as iPCCs is challenging or impossible for certain cancer types. Cancer cells may possess as-yet-undefined epigenetic aberrations, defective DNA damage responses and genetic instability-induced reprogramming checkpoints. More developed and standardized protocols for the recovery of viable cells from tumor tissues are needed to improve the reprogramming efficiency to generate these iPCCs. Alternative reprogramming methods that substitute or add to the canonical "Yamanaka transcription factors" (e.g. a cocktail of NANOG, LIN28, p53 siRNA, UTF1 and hTERT) have been shown to provide higher iPSCs reprogramming efficiency (Yu et al. 2007; Zhao et al. 2008; Park et al. 2008b) but more progress is required to reliably reprogram specific cancer cells to iPSCs and/or iPCCs. Reliable and efficient differentiation of iPSCs to specific germ layers, progenitors and terminal lineages remains a persistent problem. As cancers arise from diverse progenitor cells or de-differentiated cells in distinct tissues (Visvader 2011), differentiation protocols with higher efficiency, defined reagents and scalability are still urgently required before the entire spectrum of cancers can be modeled using iPSCs.

Some within the stem cell community have also raised concerns about increased genetic instability of iPSCs compared to other pluripotent stem cells (PSCs) or somatic cells (Hussein et al. 2011). However, recent next-generation sequencing methods have provided evidence that gene expression in iPSCs is fundamentally stable. Young et al. showed that most of the genetic heterogeneity found in iPSCs is from background mutations in parental cells (Young et al. 2012). Supporting this, Abyzov et al. showed that 50% of copy number variants present in reprogrammed iPSCs are found in parental fibroblast cells and that iPSC clones manifest genetic variants from their specifically-derived fibroblast cells (Abyzov et al. 2012). Genetic heterogeneity found in iPSCs is often acquired during extended differentiation or expansion in culture, but at a rate consistent with normal adult somatic cells acquiring spontaneous mutations during cell division (Cheng et al. 2012; Mayshar et al. 2010; Laurent et al. 2011). These studies provide clear evidence that reprogrammed iPSCs are not genetically unstable. Nevertheless, the reprogramming of adult somatic cells or cancer cells to iPSCs or iPCCs does produce global epigenomic and transcriptomic changes (Apostolou and Hochedlinger 2013), resulting in the occasional generation of partially reprogrammed "iPSC-like" cells that could be dependent on endogenous transcription factor expression (Zhang et al. 2013; Stricker et al. 2013). Completely reprogrammed iPSCs should therefore be stringently selected based on strict criteria for pluripotency and transcription factor independence (De Los Angeles et al. 2015). Still, several studies have shown that iPSCs do not exhibit greater line-to-line variation, either phenotypically or transcriptionally, compared to human ESC lines, indicating that iPSCs are not inherently epigenetically unstable (De Los Angeles et al. 2015; Guenther et al.

2010). However, the epigenetic landscape of the source cancer cell might persist after induction of pluripotency and certainly has been shown to reoccur after iPSC/iPCC differentiation. Therefore, further research on characterizing the epigenetic landscape of iPSC/iPCC-derived cells needs to be conducted to better understand the limitations of applying iPSCs and iPCCs as a cancer-disease modeling platform as well as to investigate the relationship between genetic and epigenetic changes in specific cancer types.

5 Future Perspective

Although recent advances in iPSCs have confirmed the value of this system in disease modeling and improving treatments for numerous diseases, there are still substantial hurdles precluding fulfillment of this technology's potential. Ideally, we expect to be able to utilize iPSCs to model any genetic disease (monogenic, chromosomal or complex). This promise will require a combination of gene editing technologies such CRISPR/Cas9 or TALENs, isogenic cell lines with the induction or correction of relevant mutations, as well as the generation of different mutations in the same gene in the same patient-derived iPSC or engineered hESCs (Zhou et al. 2018; Xu et al. 2018; Tu et al. 2018).

Induction of mutations in genetically complex disorders is more than theoretically possible, though after a point such experimental constructs become impractically complex to engineer. The highly variable (but typically low) efficiency of iPSC differentiation across cell lineages indicates the need for optimize cell culture conditions and differentiation protocols. Experimentation on any cells derived from PSCs must be performed on a meticulously sorted population, as the inherent ability of PSCs to divide indefinitely in appropriate culture conditions and form teratomas *in vivo* can easily complicate interpretation of assays. iPSCs generated from retroviral/lentiviral systems carry additional limitations and risks related to the unpredictable integration of genetic information into various genomic loci.

Despite these challenges, iPSC disease modeling empowers multiple research areas in translational and basic science, such as the identification and validation of therapeutic targets, preclinical efficacy and safety studies and compound screening for drug discovery and drug repurposing (Kotini et al. 2017; Doulatov et al. 2017; Crespo et al. 2017). In addition, iPSC technology can also be extended from disease modeling to cancer immunotherapy. Several groups have paved the way for the application of iPSC-technology to improve and advance cancer immunotherapy. Serwold et al. utilized PSC technology to reprogram mature T cells to T-iPSCs and re-differentiated these cells back to T cells, resulting in the generation of antigen-specific cytotoxic iPSC-derived T cells (Serwold et al. 2007). The benefit of such an approach could potentially be expanded to clinically benefit cancer patients as the unlimited and antigen-specific cytotoxic T cells could be developed to target tumor-specific antigens for enhanced cancer immunotherapy effects. In lieu of potentially extending iPSC technology from disease modeling to cancer immunotherapy, Vizcardo et al. demonstrated the generation of iPSCs derived from mature cytotoxic T cells with specificity for melanoma epitope MART-1 (Vizcardo et al. 2013). When co-cultured with OP9/DLL1 cells, these iPSCs differentiated to $TCR\beta^{+}CD4^{+}CD8^{+}$ cells with a T cell receptor (TCR) specific for MART-1 epitope, paving the way for future research on the possibility of cloning functional iPSC-derived cytotoxic T cells for cell-based cancer immunotherapy. Most recently, Kooreman et al. showed irradiated iPSCs derived from mouse fibroblasts could reduce metastatic tumor load in murine models of breast, lung, and skin cancers (Kooreman et al. 2018). These irradiated iPSCs promoted a humoral and cancer-specific anti-tumor T cell response accompanied with increased $CD11b^{+}GR1^{hi}$ myeloid cells with no observed adverse effects. These data suggests that iPSC vaccine can be potentially used in clinical immunotherapy in the future. These advances in iPSC technology demonstrate the cutting-edge potential of applying iPSCs to future cancer therapies.

In conclusion, increasingly powerful and precise genome editing technologies are enabling the study of even unusual genetic combinations in cell types with otherwise highly limited source material. The extension of iPSC technology in the application of cancer immunotherapy also proves to be extremely promising thus, we anticipate the applications of these advances to cancer biology will only increase over the coming years and facilitate development of truly personalized cancer therapies.

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Conflicts of Interest Authors declare no conflicts of interest.

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