Tumorigenic and Immunogenic Properties of Induced Pluripotent Stem Cells: a Promising Cancer Vaccine

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Abstract

Induced pluripotent stem cells (iPSCs) are mainly characterized by their unlimited proliferation abilities and potential to develop into almost any cell type. The creation of this technology has been of great interest to many scientific fields, especially regenerative biology. However, concerns about the safety of iPSC application in transplantation have arisen due to the tumorigenic and immunogenic properties of iPSCs. This review will briefly introduce the developing history of somatic reprogramming and applications of iPSC technology in regenerative medicine. In addition, the review will highlight two challenges to the efficient usage of iPSCs and the underlying mechanisms of these challenges. Finally, the review will discuss the expanding application of iPSC technology in cancer immunotherapy as a potential cancer vaccine and its advantages in auxiliary treatment compared with oncofetal antigen-based and embryonic stem cell (ESC)-based vaccines.

Keywords Induced pluripotent stem cells (iPSCs) · Somatic reprogramming · Tumorigenesis · Immunogenicity · Cancer vaccine

Introduction

A Brief History of Somatic Reprogramming

In the past, most embryologists believed only germ cells could develop into new individuals. This idea was further described by Conrad Waddington. He illustrated that the fate of cell is merely a unidirectional and irreversible process, like a ball sliding down on a random path from the hill to the valley, which cannot act in reverse [1]. However, this hypothesis was rejected by Gurdon in 1958 [2]. Gurdon transferred nuclei of intestinal epithelial cells to enucleated cells of *Xenopus leavis* resulting in healthy tadpoles. Gurdon's experiment revealed that nuclei of somatic cells were totipotent, having the ability to regenerate new offspring through somatic cell nuclear transfer (SCNT) and opened the gate for transforming adult cells into the pluripotent state. In 1996, an important milestone of success in SCNT technology was reached by the birth of Dolly, the first cloned mammal developed from an adult cell [3].

In 2006, a decade after Dolly's birth, Takahashi and Yamanaka published another instance of thrilling news. They used four transcriptional factors known as OSKM (Oct-4, Sox-2, Klf-4, and c-Myc) to artificially convert differentiated cells into a pluripotent state in vitro [4]. They referred to these cells as iPSCs. iPSCs had similar gene expression profiles to embryonic stem cells (ESCs). Therefore, their findings represented a promising alternative strategy for regenerative medicine. Since then, the development of somatic reprogramming progressed rapidly. A year later, iPSC technology was successfully applied to human cells [5, 6]. In 2011, c-Myc was replaced by L-Myc or Glis1 to remove tumorigenic potential [7–9]. Moreover, in 2014, an iPSC-generated product was underwent the first human application in a macular degeneration patient [10].

Applications of iPSCs in the Regenerative Field

Since iPSCs are characterized as easily accessible, expandable, and able to differentiate into any desired cell type, human iPSC (hiPSC) technology has created a new frontier in many fields, including: regenerative therapy, disease modeling, drug toxicity evaluation, and developmental biology [11–15].

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Compared to human ESCs (hESCs), hiPSC does not have ethical concerns; thus, hiPSC has become a preferential option for treating and modeling human genetic diseases, as well as for drug screening [16–20]. However, the exploration of iPSC applications in regenerative medicine is not without challenges. Tumorigenicity and immunogenicity of iPSCs used to be two major obstacles that frequently impeded expanding usage of iPSC technology in clinical studies [21, 22]. Concerns about the cancer formation associated with iPSCs was reported in mice generated with iPSCs through the tetraploid complementation assay, which were more inclined to develop malignant tumors than their ESC-generated counterparts [23]. Also, after successful transplantation in treating age-related macular degeneration [10, 24], the trial was halted in the second patient after genetic mutations were observed in the patient's iPSCs and its derived retinal cells [25]. Furthermore, signs of genomic instability such as chromosomal aberration, copy number variations, and single nucleotide variants were found in iPSCs, which again attracted scientific concern about their tumorigenic abilities. Although a general consensus emerged among scientists that patient-specific iPSCs are not immunogenic when performing autologous transplantations, Zhao and his colleagues demonstrated that the derivatives from murine iPSCs could trigger an immune response in syngeneic mice [26].

Currently, tumorigenic and immunogenic properties are no longer the major challenges associated with applying iPSCs in regenerative medicine. Scientists developed integration-free methods such as adenoviral vectors, Sendai viruses, plasmid vectors, and small-molecule compounds to make reprogramming process safer and more efficient [27–34]. Elimination of residual undifferentiated stem cells was also found to be vital for achieving tumor-free transplantation [35, 36]. Researchers created an in vitro selective system to ablate immature proliferating cells by introducing suicide genes into the cells [37]. In addition, by identifying and labeling undifferentiated cell markers, researchers are able to monitor remnant immature cells in vivo [38]. Moreover, recent clinical studies showed that the rejection of grafts have not been observed in patients for at least short-term period without using immunosuppression methods (Table 1). However, it is still necessary to investigate the mechanisms behind the tumorigenicity and immunogenicity of induced pluripotent cells in order to improve understanding of this technology. More tumorigenicity and immunogenicity studies could enable iPSC technology to expand beyond the traditional realms of drug screening, disease modeling, and cell/tissue regeneration into other fields like cancer immunotherapy.

The Tumorigenic Potential of iPSCs (Fig. 1)

The Shared Features Between iPSCs and Neoplastic Cells

The most evident similarity between iPSCs and tumor cells is their capacity to renew themselves, as well as their capacity for infinite cellular proliferation. To adapt to this feature, unlike the growth of somatic cells, which can be inhibited as they make contact with other cells, tumors and iPSC colonies can somehow surpass this restriction [41]. Second, studies have shown that iPSCs and cancer cells also have high telomerase activity, and thus can promote telomere elongation against age-caused senescence [42, 43]. Moreover, these two cells types also present a similar metabolic pattern, which is dominated by glycolysis in response to a rapid proliferation rate [44, 45].

The fundamental reason why iPSCs share multiple phenotypes with cancer cells is that both cell types express increased levels of oncogenes [46, 47]. Researchers have reported not only similar overall gene expression profiles, but also parallel microRNA signatures and epigenetic status between iPSCs and neoplastic cells [48, 49]. By comparing RNA-sequence data of iPSC colonies with a list of selected cancer-related genes, Kooreman and his colleagues revealed a large overlap in gene-expression profiles shared by various types of cancer cells and iPSCs [50].

The Function of Pluripotent Genes in Carcinogenesis

Pluripotent stem cells (PSCs) including ESCs and iPSCs have conserved gene expression networks with cancer cells [51].

Table 1 The clinical studies of iPSC usage in regenerative medicine

| The type of disease	Cell type	Function after transplantation	Immune response	Immunosuppression methods	Reference
Parkinson's disease	Autologous iPSC-derived Dopaminergic progenitor cells	Improved	Negative	Absent	[39]
Macular degeneration	Autologous iPSC-derived pigment epithelial (RPE) cells	Improved	Negative	Absent	[40]
Heart failure	Allogenic iPSC-derived cardiomyocytes (hiPSC-CM)	Ongoing	Assessing	Assessing	NCT 03763136
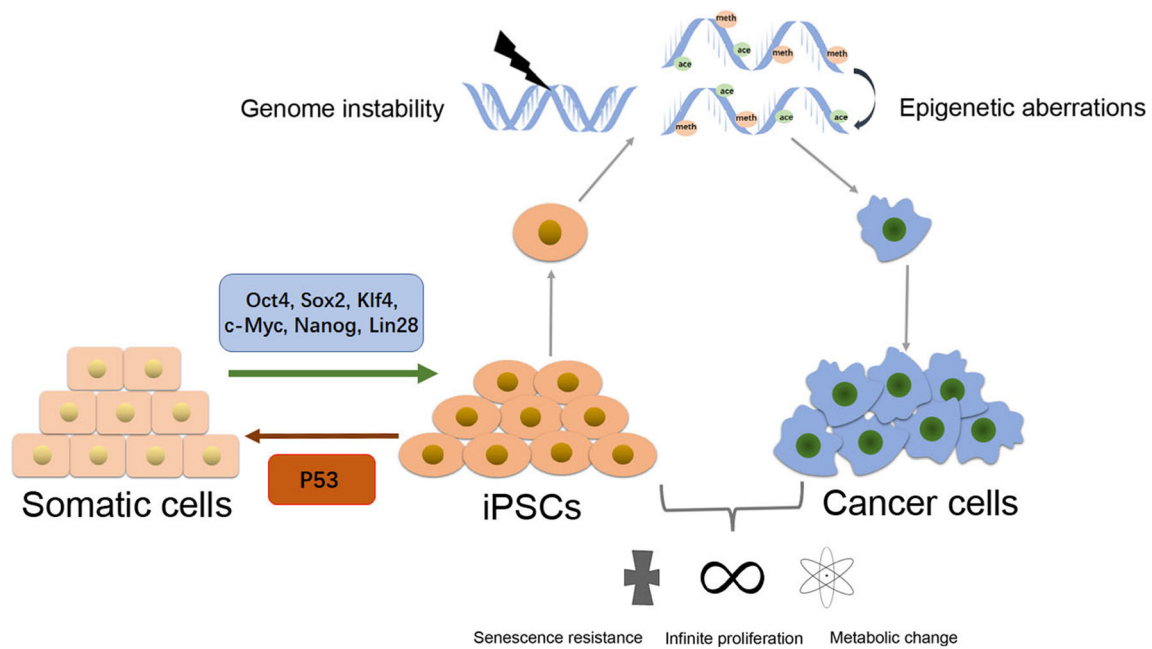
Fig. 1 The adverse effects of pluripotent genes and P53 in iPSC reprogramming and tumor formation. While the core pluripotent factors like Oct4, Sox2, Klf4, c-Myc, Nanog and Lin28 are promoting the somatic reprogramming process, one of the vital tumor suppressor gene - P53 acts like a barrier to impede this. Therefore, in order to achieve the final goal, p53 is required to be silenced at the early stage of reprogramming. However, this could lead to genome instability within the iPSC and eventually resulting in tumorigenesis. Abnormal epigenetic modification accumulated in the iPSCs from reprogramming to prolonged culture also contributes to the risk of tumorigenic potential

Many of these shared proteins are also defined as oncofetal antigens. These tumor-associated proteins are reported to appear during embryonic development, but disappear in adult somatic cells and show themselves again during cancer formation [52]. For instance, a-fetoprotein (AFP) is highly expressed during fetal generation and normally remains almost undetectable when people reach adulthood. When it is ectopically expressed in an adult, it is usually associated with hepatocellular cancer or germ cell tumors. Thus, it has been used as biomarker in oncology for detecting these two kinds of tumors [53].

It should be noticed that the genes involved in either the original Yamanaka reprogramming cocktail or other version, in which c-Myc and Klf4 are replaced by Lin28 and Nanog, are largely transcription factors with potent oncogenic properties [4, 6]. C-Myc is a well-defined oncogene expressed by many different types of cancers [48, 54]. In 2007, Takahashi et al. identified three major functions of c-Myc in iPSCs, including inhibiting cell differentiation, promoting cellular proliferation, and amplifying the activities of Oct4, Sox2, and Klf4 [5]. Augmented expression of c-Myc can influence thousands of genes; most upregulated genes play positive roles in cell growth, whereas downregulated genes are largely associated with cell cycle arrest, cellular adhesion, and intracellular communication [55]. Chimaeras obtained from conventional iPSC clones mostly developed tumors within one year, and approximately one fifth among them showed the reactivation of the c-myc

genes [56]. On the contrary, mice developed from a modified protocol that excluded Myc retrovirus exhibited negative results in tumor formation [57]. It is very obvious now that c-Myc acts as a "double-edged" sword. C-Myc can significantly boost the efficiency of iPSC generation, but in contrast, it also hinders clinical usage by increasing tumorigenicity in iPSCs and their derivatives.

In addition, other core pluripotent factors also play critical roles in both pluripotency and oncogenesis. It has been revealed that the ectopic expression of Oct4 in somatic cells, such as epithelial cells and intestinal cells, led to the development of malignant features and dysplastic growth [58]. From normal cervical cells to invasive cervical cancer cells, as the invasiveness of cervical cells increases, Oct4 protein expression level also increases. Evidence has shown that Oct4 overexpression facilitates carcinogenesis by inducing miR-125b expression to inhibit cancer cell apoptosis [59]. Oct4 is also required for maintaining the stemness of cancer cells in multiple cancer types [60–64]. Sox2 is able to form heterodimer with Oct4 to play a delicate role in regulating the pluripotent state of pluripotent stem cells. In human colorectal cancer cells, Sox2 induces a stem-like state with downregulated CDX2 expression level. CDX2 is the epithelial marker of the intestine and its low expression level associated with poor patient prognosis [65].

Klf4 is a transcription factor that has diverse functions in both promoting oncogenesis and suppressing tumor formation. A study showed that overexpression of Klf4 in a chosen

colon cancer cell line reduced colony formation not because of apoptosis induction, but due to its role in mediating p53-dependent cell cycle arrest [66]. However, evidence also indicated that Klf4 could promote the expression of cancer stem cell-related markers and mesenchymal markers in colon cancer cells [67]. Similarly, in hepatocellular carcinoma cells, Klf4 can convert regular cancer cells into a cancer stem cell-like phenotype by positively regulating the expression of EpCAM and E-CAD [68]. Researchers also confirmed that Klf4 acted as a potent oncogene in breast cancer development via facilitating cell invasion, migration, and the maintenance of stem cell features [69]. As a stem cell marker, Nanog was demonstrated to be critical for somatic cells to surpass the intermediate dedifferentiated state to reach ultimate pluripotency in the reprogramming process. Also, in the embryo, Nanog-deficiency caused the inner cell mass to be maintained in the pre-pluripotent phase [70]. Nevertheless, Nanog seems to actively participate in generating tumor-initiating stem-like cells (TICs) through metabolic pathways. ChIP-seq data showed that Nanog regulated mitochondrial-related metabolic genes to sustain the abilities of self-renewal and drug resistance in TICs [71]. According to genome-wide location analyses, Oct4, Sox2, and Nanog all bind to their own and each other's promoters to form a sophisticated autoregulation circle. Lin28 is overexpressed in multiple cancer cell lines, and acts as a potent oncogene. The Let-7 family is a group of microRNAs that suppresses tumorigenesis and induces differentiation in ESCs. It has been demonstrated that Lin28 can block the maturation process of let-7 precursors to facilitate malignant transformation in vitro [72]. Studies showed that Lin28 could directly bind to Oct4 mRNA, and thereby regulate the expression of Oct4 at a post-transcriptional level in hESCs [73]. In epithelial ovarian cancer, Lin28 and Oct4 have a synergic effect in both promoting tumor cell survival and creating a favorable microenvironment for cell growth [74, 75].

Negative Effects of p53 in Induced Pluripotency

P53 is a transcriptional factor that is mutated in more than half of all cancer types. As the "genomic guardian," the p53 gene plays an essential role in maintaining genomic stability in mammalian cells [76–78]. P53 can directly influence the expression of hundreds of genes. For example, p53 can activate cancer suppressor genes like p21, MDM2, GADD45, PERP, NOXA, and CYCLING, and also suppresses expression levels of oncogenes like MAP4 and NANOG. When encountering various stresses (genotoxic, oncogenic, and oxidative stresses), p53 is stabilized by multiple post-translation modifications that subsequently lead to cell cycle arrest [79–85]. Next, the p53-dependent DNA repairing process is activated to protect the stability of the genome. However, if the damage is already catastrophic, then p53 facilitates senescence and

apoptosis to eliminate a potential tumorigenic outcome [76, 86–88]. In the absence of stress, the activity of p53 protein remains at a low level due to inhibition by MDM2 and MDMX [89–92]. MDM2 is an E3 ligase that forms a complex with p53 through protein-protein interaction, which in turn leads to p53 degradation [93].

As we mentioned before, factors contained in a reprogramming cocktail are oncogenic, and often ectopically expressed in many tumors. Thus, during iPSC generation, p53 has become a major obstructive factor that hampers the efficiency of iPSC development [92, 94]. It has been reported that p53 activation in cells undergoing reprogramming reduces the expression level of Nanog, which is critical for maintaining pluripotency [94]. Also, it is not surprising that scientists found that silencing p53 during cellular reprogramming would largely improve cell-generating efficiency [53, 90, 95–97]. Oct4 and ZSCAN4 have been proven to promote reprogramming efficiency by inhibiting p53 [98, 99]. Moreover, suppressing the expressions of the p21 and PUMA gene, which are downstream of the p53-dependent gene network, can also increase the frequency of reprogramming [100, 101]. All of the above evidence indicates that transient p53-suppression during the early stage of iPSC reprogramming may be crucial for successfully generating mature colonies in the future.

Owing to the critical role of p53 in protecting genomic stability and its negative effects in either inducing or maintaining the pluripotent state, a serious concern was raised for genomic instability in iPSCs. Accordingly, the results from independent groups indicated that the induced reprogramming processes are responsible for many genetic mutations. Ji et al. showed that over 70% of point mutations were gained from somatic reprogramming [102]. In addition, Sugiura and his group performed whole genome sequencing (WGS) analysis to unravel the enigma. They discovered that hundreds of point mutations occurred immediately after the onset of iPSC reprogramming. Moreover, they compared the point mutation profiles between ESCs and iPSCs under identical conditions, and found that the rate of point mutations in iPSCs was much higher than the rate in ESCs [103]. This evidence suggests that p53 silencing during the early dedifferentiation stage may be responsible for genetic mutations induced by somatic reprogramming and, therefore, for the possible risk of oncogenesis.

Genome and Epigenetic Aberrations in iPSCs

The genomic instability of iPSCs has been well summarized in a review written by Yoshihara et al. [104]. Genomic abnormalities include chromosomal aberrations, copy number variations, and single nucleotide variants. The first chromosomal aberration in iPSCs was observed in 2010 [105]. Trisomy 12 is most commonly observed in both ESCs and iPSCs

[106–108]. Since chromosome 12 contains genes involved in cell cycle regulation and harbors pluripotent gene Nanog [105], this chromosome may provide selective advantages among mutant cell subpopulations. Additionally, it had been reported that prolonged culture can cause a gain in the 12p region, which is also a hallmark of testicular tumors [109, 110]. Several studies later determined that amplification of 20q11.21 was the most recurrent mutation in iPSC lines, and 20q11 duplication was shown in many tumors as well [107, 108, 111, 112]. This region enriched pluripotent-related and anti-apoptosis associated genes, such as DNA methyltransferase 3B (DNMT3B), inhibitor of DNA binding 1(ID1), and BCL2-like1 (BCL2L1). Laurent et al. demonstrated that copy number variation types can be changed through passaging. During early passage of human iPSCs, the deletion of tumor-suppressor genes appears frequently. However, during late passage, the duplication of oncogenic genes increased [108].

Although the major epigenetic profiles of iPSCs are similar to those of ESCs, these two pluripotent cell types still exhibit significant differences, such as aberrant silencing of imprinted genes and DNA methylation patterns. Genomic imprinting is a preferential gene expression pattern that is inherited from either maternal or parental germ cells. Abnormal imprinting has significant impacts on brain function, and is associated with various diseases [113]. The overall expression of imprinted genes in iPSCs is rather stable, but numerous iPSC lines with aberrant genomic imprinting still remain. Some of these errors may due to abnormal DNA demethylation in the promoter regions [114]. The DNA methylation abnormalities of iPSCs mainly result from two aspects. One is that iPSCs keep the epigenetic memory from their somatic sources [115, 116]. Researchers investigated the organ-specific DNA methylation in six isogenic organ-derived hiPSCs. They found that iPSCs produced by the fetal brain maintained distinguishing DNA methylation marks, which were sufficient to provide differentiation preference into neural derivatives [116]. The other major contributing factor of aberrant DNA methylation is the reprogramming process itself [117]. During the reprogramming process, Oct4 overexpression was proven to result in off-target gene activation and epigenetic aberrations [118]. The types, locations, and amount of DNA methylation errors could be affected by different combinations of reprogramming factors [119]. Interestingly, some DNA methylation differences between hiPSC and hESC could be erased by prolonged culturing [117, 120], while others seem to be very "stubborn," and will pass through generations regardless of pluripotent state or differentiated state [121]. Moreover, the alterations in DNA methylation within cancer-specific gene promoters were also observed in iPSCs, suggesting a positive correlation between induced pluripotency and carcinogenesis [49].

# The Immunogenicity of iPSCs and their Derivatives

The Lasting Debate about Immunogenicity of iPSCs and Their Derivatives

The invention of iPSC-generation technology has long invigorated the regenerative medical field due to the hope that iPSCs will not trigger an immune response in syngeneic recipients. However, this idea was dampened by the discovery that iPSCs and their differentiated products might be immunogenic to genetic-matched recipients. In 2011, Zhao's group published a study in *Nature*, in which they transplanted ESC-derived teratomas, as well as teratomas derived from iPSCs, into syngeneic mice in order to assess their immunogenicity. Surprisingly, unlike ESC-derived teratomas, which caused no evident immune rejection, iPSC-derived teratomas were mostly immune-rejected and displayed T cell infiltration with apparent tissue necrosis. Furthermore, Zhao et al. argued that several genes expressed specifically in iPSCs, such as Zg16 and Hormad1, were responsible for the immune rejection [26]. Nevertheless, Araki et al. demonstrated that when iPSCs were differentiated through chimera formation in vivo, the iPSCs would not be immunogenic to genetic matched recipients [122]. Similarly, Guha and his group discovered that neither differentiated nor undifferentiated murine iPSCs activated an immune rejection after transplantation. They established embryoid bodies (EBs) and representative cell types from three germ layers (endothelial cells, hepatocytes, and neurons) of murine iPSCs, and analyzed all their immunogenicity after grafting. According to their work, iPSCs and their derivatives did not elicit evident T cell proliferation or antigen-specific immune responses [123]. Preclinical studies in non-human primates demonstrated that transplanted autologous iPSC-derived neural cells survived well in those animals' brains without provoking an evident inflammatory response [124, 125]. These results suggested that grafts generated from autologous iPSCs could be safely applied as regenerative resources without immunogenicity concerns.

Factors Influencing Immunogenicity in iPSCs and Their Derivatives (Fig. 2)

Although knowledge of whether iPSCs and their derivatives are immune tolerant to syngeneic recipients is incomplete, according to recent studies, the immunogenicity of iPSCs and their products is contributed to by several factors. First, remaining undifferentiated iPSCs are one of the major reasons for grafts' rejection after transplantation. De Almeida et al. demonstrated that iPSC-derived endothelial cells could

Fig. 2 The influential factors of immunogenicity within iPSC-derived products. Five reasons for iPSC derivatives to provoke immune response in genetic matched recipients. A) undifferentiated iPS cells within differentiated products. B) specific cell types like muscle cells. C) extended passage in vitro. D) the microenvironment of transplanted site. E) de novo mutations of mitochrial DNA
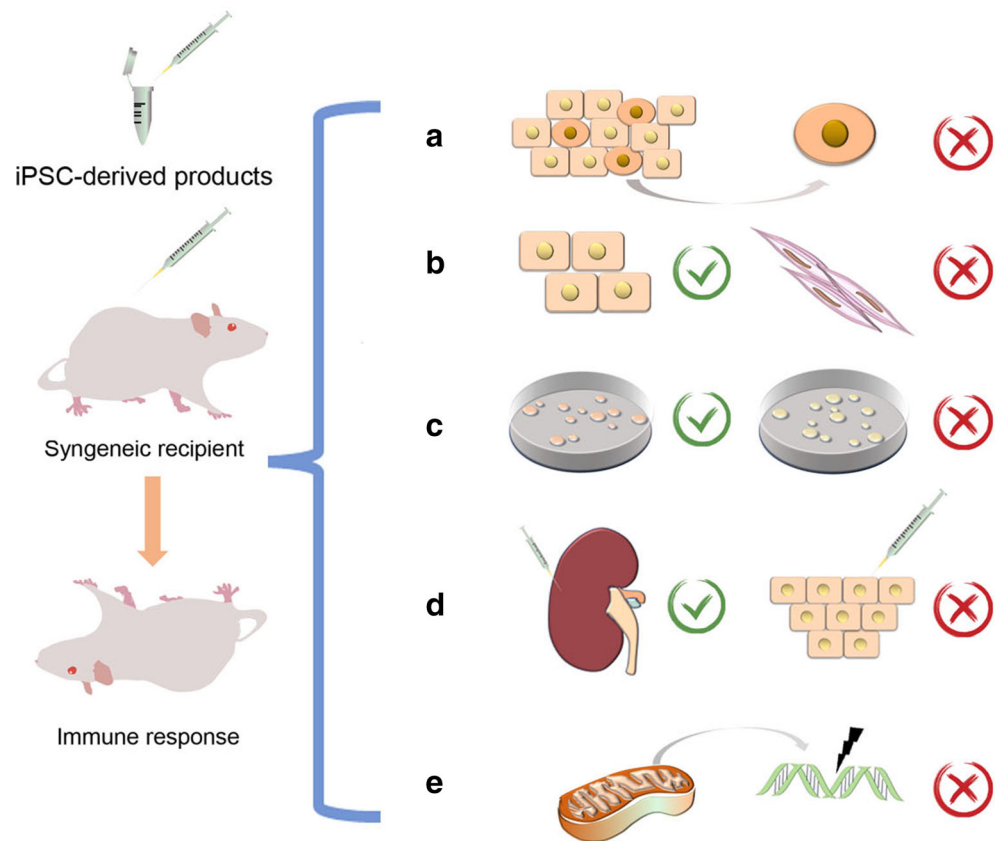
survive for a long period when mediated by increased IL-10 after transplantation, just like aortic endothelial cells [126]. On the contrary, undifferentiated iPSCs were highly immunogenic to syngeneic recipients, and attracting lymphocyte infiltration and increasing the secretion of interferon-r (INF-r), granzyme-b, and perforin [126]. The mechanism behind this phenomenon is that the iPSCs tend to differentiate into cells that express gamete-associated proteins (GAPs). Since these proteins are originally expressed in gonads and not tolerated by T cells in the peripheral blood, GAPs are responsible for the immune rejection of undifferentiated iPSCs. Accordingly, iPSC-derived hematopoietic progenitor cells that no longer express GAPs are able to subsist long-term in syngeneic recipients [127]. Second, different cell types derived from the same iPSC can have different outcomes. Zhao et al. used a humanized mouse model to simulate the human immune environment. Interestingly, while human iPSC-originated smooth muscle cells elicited an intensive immune response, retinal pigment epithelium derived from human iPSCs had immune privilege even when transplanted in non-ocular sites. This result suggested that distinctive cell types exhibit distinguished antigens, which cause different immunogenic responses [128]. Similarly, iPSC-derived cartilages and neural

crest stem cells were identified as having low immunogenicity due to the limited expression of MHC-class molecules [129, 130]. Third, the immunogenicity of iPSC-derived grafts is determined by the microenvironment of the transplanted site. iPSCs and their terminal differentiated products survived well when transplanted in the kidney capsule, and caused no evident immune reaction. However, iPSC-derived cells were rejected when injected into subcutaneous tissue or an intramuscular site. Further evidence showed that this discrepancy was caused by the absence of mature dendritic cells (DCs), since co-transplanted genetic-matched DCs with iPSC-originated cells activated a strong immune response in the kidney capsule [131]. In addition, extended passaging in vitro also contributes to iPSC-provoked immune responses. A study showed that early passage of iPSCs generated from Sertoli cells could form more teratomas in vivo with less immune cell infiltration, tissue damage, and necrosis than prolonged-passage iPSCs [132]. Finally, de novo mutations of mitochondrial DNA could be another factor that elicits immune recognition and rejection. Since mitochondrial DNA has less reliable repair mechanisms, the nonsynonymous mutations could occur during the reprogramming stage or long-term culture, and ultimately be

accumulated into differentiated cells. Some of these mutants produce neoantigens and lead to immunorecognition and rejection [133].

Possible Application of iPSC Technology in Developing Cancer Vaccine Development

The interaction between pluripotent stem cells and cancer immunotherapy has been studied for over a century. Schöne et al. uncovered the fact that immunization with embryonic tissue encouraged rejection of transplanted tumors in mice [134]. Taken one step further, Fibiger and his colleagues used fetal skin to immunize mice and found that tumor growth and metastasis were efficiently prevented [135]. Numerous studies have demonstrated that immunization with fetal materials could not only inhibit the growth of transplanted tumors, but also showed a protective effect against tumor formation when exposed subjects were exposed to viral or chemical carcinogens [136]. Later studies showed that hESC-elicited monoclonal antibodies could bind to various cancer cells [137–139]. Also, ESC vaccination in mice would promote a significant anti-tumor effect with increased lymphocyte proliferation and cytokine secretion [140]. Moreover, much evidence supported that potent T cell responses could be induced by oncofetal peptide-based cancer vaccines [141–144]. A wide range of oncofetal peptide-based vaccines have entered into the preclinical phase, and some have even reached the clinical trial phase [145–148].

While much progress has been made, many problems are still waiting to be solved. An oncofetal peptide-based vaccine can only target one antigen. Since tumor cells are highly heterogenic and present with a high mutation rate, this kind of vaccine may not be able to provide durable and effective protection for patients. For instance, MAGE-A3 is overexpressed in various cancer types and used as a cancer vaccine target. However, in non-small cell lung carcinoma, this adjuvant treatment failed to improve cancer-free survival compared with the placebo group. Thus, further investigation of the MAGE-A3 cancer vaccine had been terminated [147]. Similarly, the glypican-3-targeted vaccine was halted in a phase II clinical trial of hepatocellular carcinoma. In this study, although significant numbers of glypican-3-specifiic cytotoxic T lymphocytes (CTLs) was found in patients, they could not offer a protective effect against recurrent tumors, since glypican-3 was no longer expressed by those tumors [148]. For an ESC-based vaccine, it is hard to exclude immune responses generated by incompatibility of the MHC antigens between donors and recipients, which reduces the EPC-based vaccine's clinical applicability [149, 150].

In this context, generating a cancer vaccine from autologous cells would be a reasonable direction. Some work has been done to investigate the possibility of generating

an iPSC-based cancer vaccine and autologous tumor cell-based vaccine. Li et al. created the cancer vaccine from a TZ1 human iPSC line to treat transplanted colon cancer in mice. However, this vaccine failed to provide evidence of an anti-tumor effect despite inducing significant numbers of IFN-r and IL-4-producing splenocytes [149]. After this study, researching a cancer vaccine generated from iPSCs seemed to enter a dormant period. During this time, scientists paid more attention to the possibility of an autologous cancer vaccine. An autologous melanoma vaccine was evaluated in patients as an adjuvant or active treatment. This vaccine was shown to stimulate strong anti-melanoma CD4+ T cell activity, which is associated with improved survival [151]. Moreover, given the vital role that cancer stem cells (CSCs) play in sustaining tumor growth and causing relapse after therapy, vaccines generated from different types of CSCs were also proven to display effective tumor immunity in respective cancers [152, 153].

In 2018, Kooreman and his group published their work in *Cell Stem Cell*. They updated the early version of the iPSC-based vaccine by adding an immunostimulatory adjuvant, CpG oligodeoxynucleotide, into the vaccine. CpG oligodeoxynucleotide is a TLR9 agonist that can facilitate the maturation of antigen-presenting cells (APCs). They also irradiated iPSCs before injecting them into mice to avoid teratoma formation. This study found that iPSC-based vaccine injection could induce specific antibodies as well as CD4$^+$ and CD8$^+$ T cells. Furthermore, they observed an increased level of CD8$^+$ T cells in recipient mice, whereas the level of CD4$^+$CD25$^+$FOXP3 Treg cells decreased. Mice immunized with an iPSC-based cancer vaccine could reject transplanted breast cancer, melanoma, and mesothelioma cells. In addition, T cells transplanted from immunized mice to naïve mice could transfer this tumor inhibitive function to recipient mice. Although the researchers failed to demonstrate protective effect of in established melanoma, probably due to the immunosuppressive environment caused by cancer development, iPSC plus CpG vaccine successfully prevented tumor relapse after removing the primary tumor [50]. These results may suggest that the tumorigenic risk and immunogenicity of iPSCs can be utilized as advantages to generate iPSC-based cancer vaccines, which may exhibit potential usage as auxiliary therapy after surgery. Another parallel study demonstrated that iPSC had similar gene expression patterns with lung adenocarcinoma stem cells and could provoke anti-tumor immunity in humanized mice model [154]. Very recently, Gąbka-Buszek et al. combined the whole-cell melanoma vaccine with stem cells, and demonstrated the increased effectiveness of the mixed cells in anti-tumor immunity. They mingled Hyper-IL6 (H16) gene-modified tumor cells with either melanoma stem-like cells (MSCs) or iPSCs to construct two types of "mixed

vaccines." H16 is a fusion protein containing IL-6 and is a potent activator in many important signaling pathways, including JAK1/STAT3, MAPK, and PI3K. In modified tumor cells, H16 serves as a molecular adjuvant that blocks the formation of Treg cells, and promotes dendritic cell maturation and antigen presentation [155]. Previous studies and clinical trials showed a high response rate, extended disease-free survival (DFS), and long-term overall survival (OS) in patients treated with the H16 modified tumor cells [156–158]. In this study, although immunization with either modified cells alone or "mixed vaccines" could both significantly inhibit tumor growth and increase survival in mice, vaccines containing stem cells demonstrated a higher immune response in the vaccination site and tumor microenvironment. In addition, the most effective DFS and OS extensions were achieved with the vaccine containing miPSCs [159]. This work verifies the hypothesis that enrichment of a whole-cell vaccine with stem cells increases the anti-tumor potential of the vaccine and provides us another angle for creating more effective cancer vaccines.

Future Research Directions

For more than a decade, studies about the applications of iPSCs have mainly focused on the regenerative field. In this context, carcinogenesis and immunogenicity are two major obstacles that scientists should overcome. Therefore, plenty of researchers are focusing on either developing new reprogramming methods, or creating mutation screening protocols to circumvent tumor formation and immune response after transplantation. Nevertheless, these obstacles may provide iPSC unique characteristics that allow it to become a promising strategy in cancer immunotherapy. Moreover, an iPSC-based vaccine can easily compensate for disadvantages generated by oncofetal antigen-based and ESC-based vaccines. First, as a whole-cell based cancer vaccine, iPSCs can provide multiple oncofetal antigens, which can cover a wide range of tumor types and compensate for the heterogenicity within tumors. Second, an autologously developed iPSC vaccine can resolve the problem of MHC incompatibility, and therefore reduce the possibility of an immune response to MHC-related proteins. An iPSC vaccine can focus on the oncofetal peptides shared by pluripotent cells and neoplastic cells. Since using iPSC alone cannot induce a powerful anti-tumor immune response, future studies should focus on investigating safe and potent immunostimulatory adjuvants to help iPSCs elicit anti-tumor immunity. The other possible direction may be evaluating the synergic effects of combining iPSCs with other cancer-specific cells in a vaccine.

Compliance with Ethical Standards

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