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Induced pluripotent stem cells (iPSCs) based approaches for hematopoietic cancer therapy

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Abstract
Induced pluripotent stem cells (iPSCs) are reprogrammed from somatic cells through numerous transcription factors. Human induced pluripotent stem cell approaches are developing as a hopeful strategy to improve our knowledge of genetic association studies and the underlying molecular mechanisms. Rapid progression in stem cell therapy and cell reprogramming provides compelling reasons for its feasibility for treating a wide range of diseases through the replacement of autologous cells. Continuous failure in embryonic stem cells (ESC) production and the dependency of iPSC on ectopic genes may be due to the inability to maintain the stability of the endogenous gene systems which are essential for creation of pluripotency state. With recent developments in the genome processing and human tissue culturing approaches as well as xenotransplantation, bioengineering, and genome editing, induced pluripotent stem cells offer the new opportunities for the study of human cancers. Most hematopoietic malignancies are originated from cells that are functionally heterogeneous and few of them are responsible for maintaining tumor state. The naming of these cancer stem cells are due to the quality characteristics of normal tissue stem cells, such as self-renewal, long term survival, and the ability to produce cells with more differentiated properties. The aim of present study was to focus on the recent progresses in the application of stem cell-based hematopoietic cancer, and to assess the benefits of treatment, opportunities, and shortcomings that can potentially help improve future efforts in experimental and clinical studies.
Keywords: Gene therapy, Hematopoietic cancer, Induced pluripotent stem cells

Introduction

The human body comprises of over two hundred different types of cells that form tissues and organs and provide all the essential functions for survival and reproduction (1). All of cells in the body, both somatic (of all three germ layers) and germ cells, originate from the embryonic pluripotent cells (2). In 1961, for the first time, Till and McCulloh (3) introduced self-renewal properties in any living cells as one of their important breakthrough. They influenced the mice with lethal radiation doses and then injected bone marrow cells in mice. They found that the main reason of survived of the mice was these cells formed clumps due to cell cloned from them (4, 5). In 2006, Japanese researchers at Kyoto University recognized conditions that allowed specialized adult cells to be genetically "reprogrammed" to accept a stem cell-like state (6). These specialized cells, which are now called induced pluripotent stem cells (iPSCs), , were reprogrammed to an embryonic stem cell-like state via introducing important genes to maintaining the critical properties of embryonic stem cells (ESCs) (7). After this discovery, researchers have improved the generation techniques of iPSCs and created powerful new pathways to de-differentiate cells, which their growth and developmental conditions was already determined (8-10). The main focus of worldwide investigators and clinicians is on the potential use of iPSCs as a helpful tool for disease modeling, drug development, and regenerative medicine

(11-13). Furthermore, the ethical issues related to the ESCs generation are not applied to iPSCs, proposing a non-controversial strategy to create pluripotent cells using existing non-pluripotent cell lines which can be transplanted into the patients without the concern of immune rejection (autologous graft) (14, 15). However, there are many challenges in pre-temporary iPSC tests, one of which is teratoma development or tumorigenesis risk if they are inserted in sensitive sites such as promoters and host gene enhancers (16).

Experimental strategies for iPSCs generation

One strategy to achieve **iPSCs** generation is nuclear reprogramming, a stable alteration in the nucleus of a mature cell, which can then be preserved and replicated as the cell divides through mitosis (17). This strategy is implemented using techniques such as fuse somatic cells with ESCs, somatic cell nuclear transfer (SCNT), and altered nuclear transfer (ANT) (18, 19). The nuclear reprogramming approach involves the use of mature "somatic" cells from an adult and the introduction of genes that encoding critical transcription factor proteins that themselves regulate the function of other important genes in the early developmental stages of the fetus (20, 21).

In the previous studies, it was found that only four transcription factors (Oct4: Octamer binding transcription factor-4, Sox2: Sex determining region Y-box2, Klf4: Krupple like factor 4, and c-Myc) were required for reprogramming of mouse fibroblasts to an embryonic stem cell–like state by forcing them to express important genes for maintenance of the defining properties of ESCs (22-24). In 2007, two different research teams reached a new milestone through deriving iPSCs from human cells and using the original four genes containing Oct4, Sox2, Nanog, and Lin28 (25, 26). Since then, researchers have achieved the production of iPSCs from somatic tissues of the rat and monkey (27-29).

Several methods have been considered for improving the effectiveness of the reprogramming and reducing the potentially harmful side effects of the reprogramming process (30). Since the retroviruses used to deliver the four transcription factors in the earliest studies can be potentially mutagens, researchers are not certain about whether all four factors are absolutely essential (23, 31, 32). Specially, the *c-Myc* gene is known to increase the rate of tumor growth in some cases, which negatively affects iPSC efficiency in transplantation treatments (27, 33). For this purpose, researchers proposed a three-factor approach using Oct4 with the orphan nuclear receptor Esrrb and Sox2, and they were able to transform mouse embryonic fibroblasts into iPSCs. Similarly, other reports have indicated that c-Myc has not a functional role for direct reprogramming of mouse fibroblasts (34, 35). Studies have further reduced the number of essential genes for reprogramming, and investigators continue to recognize of chemicals that can replace or increase the efficiency of transcription factors in this procedure (36, 37). These successes continue to inform and facilitate the reprogramming process, thus advancing the field toward the production of patient specific stem cells for clinical application. However, the way through which transcription factors transfer to the somatic cells is critical to their potential use in the clinic (38).

IPSCs in cancer therapy: opportunities and challenges

Normally derived iPSCs from patient tissues can mainly be employed for tumor regeneration or treatment of the affected tissues. In regenerative medicine, numerous tissues can be produced by using iPSCs (2, 39). IPSCs therapy may be a good alternative for damaging approaches such as chemotherapy, radiotherapy, or surgical treatment.

However, regenerative therapy mediated by human iPSCs needs strong in vivo evidence from iPSC-derived tissues (19, 40).

Now, only a few types of human iPSC-derived cells (e.g. hepatocytes) have been effectively assessed in vivo (41, 42). In addition to the direct role of iPSCs in cancer treatment, they can be utilized to screen novel anticancer drugs. Derived iPSCs from differentiating cancer tissue generate cell types that may be more biologically linked to human tumors and be more suitable for drug screening methods. IPSCs are also better candidates than other stem cells for evaluation of the toxicities of antitumor drugs (25, 37, 43).

Similar to most chemotherapy components, stem cell therapy using a single agent commonly cannot eliminate tumors. Therefore, a desired drug combination should be rationally generated and selected (44, 45). Many of these combination therapies have been tested to improve treatment durability. For example, chemotherapy combined with interferon (IFN)-beta immunotherapy, by using a pro-drug/suicide gene system, has displayed synergistic therapeutic effects in human colorectal cancer (44, 46). In 2011, Zielske et al., found that irradiating tumor cells could induce production of factors that stimulate stem cell invasion and increase the number of stem cell in tumors (47).

There are three possibilities for tumors in which cancer stem cells play a critical role. First, the mutations in the normal stem cells or progenitor cells and transformation of them into cancerous stem cells which can lead to the growth of the primary tumor. Second, many of the primary tumor cells may be killed during chemotherapy, but if the cancerous stem cells are not eliminated, they become cancer-resistant stem cells and may result in tumor recurrence. Third, the cancer stem cells may emigrate to distal positions from the primary tumor and cause metastasis (48). For these reasons, most previous researchers paid special attention to the pluripotent stem cells that might be highly tumorigenic. However, recently it is shown that iPSCs is safer for clinical use than ESCs and other stem cells (49). However, various strategies are used to minimize the risk and possibility of neoplastic transformation.

In the first step, undifferentiated pluripotent stem cells, which are potentially tumorigenic, can be separated from clinical approaches using antibodies that focus on specific surface-displayed biomarkers. Stem cell differentiation downregulates expression of these biomarkers (50).

Second, directed differentiation of iPSCs involves monitoring of the expression of the specific differentiation genes. Successfully differentiated cells can be recognized and screened by using recombinant reporter proteins. Green fluorescent protein (GFP) and similar proteins work well as reporters of differentiated against undifferentiated cells (51). Third, antibody-guided toxins or toxic antibodies can kill the undifferentiated pluripotent stem cells through immune pathways. For instance, monoclonal antibodies against claudin-6, a surface biomarker for undifferentiated pluripotent ESCs and iPSCs, can guide immune-toxins to these stem cells for targeted and selective killing (52).

Fourth, undifferentiated pluripotent stem cells can be removed from cytotoxic components, which can be utilized to kill targeted and selectively pluripotent stem cells that can progress into tumors (53).

Fifth, potentially tumorigenic pluripotent stem cells can be transformed with suicide genes for sensitization towards pro-drugs. For this purpose, the enzyme/pro-drug cancer therapy strategies can be improved to kill undifferentiated pluripotent stem cells (54) Finally, tumorigenic pluripotent stem cells can be eradicated through self-induced transgenic expression of recombinant human DNases (55). These

strategies can protect a large population of stem cells against tumor transformation.

Application of iPSCs in regenerative medicine for cancer therapy

It is proven that reprogramming of human primary cancer cells is very difficult. However, there have been relatively few reports demonstrating iPSCs production for successful reprogramming of tumoric human cells. The generation of novel iPSC and reprogramming cancer cells are thought to be faced with difficulties such as reprogramming-triggered cellular senescence, tumor-specific genetic mutations, epigenetic modifications, genomic instability, and accumulation of DNA damage. In spite of these difficulties, several researchers have reported the generation of new iPSC lines from present cancer cell lines. Reports of iPSC lines resulting from human cancer cell lines are summarized in Table I, which presents a range of cancers such as leukemia (AML) (56), breast cancer (57), retinoblastoma (58), colon cancer (44), Lliver cancer (59), hematologic and solid malignancies (60), melanoma (61), myelomonocytic leukemia (62), and glioblastoma (63). Based on these studies, reprogramming of cells with human cancer cell lines is not an impossible task. However, certain types of cancers may be commonly used against reprogramming factors and the combination of factors should be carefully selected depending on the type of cancer cells that are attempting to reprogram. Nevertheless, reports of iPSCs generated from human primary malignant cells are few and limited to certain cancers, especially leukemia. Hu et al., were successful in reprogramming primary human lymphoblasts using transgene-free iPSC technology to ectopically express OSKM, LIN28, NANOG, and the SV40 large T gene from a $BCR^{-}ABL^{+}$ CML patient (64). Kim et al., reported generation of single iPSC-like line in the parental pancreatic ductal adenocarcinoma (PDAC) cancer cultures that contained KRAS G12D mutation (65). The rarity of successful studies demonstrates the difficulty of reprogramming primary cancer cells to iPSCs. However, technical limitations, such as complications in maintenance primary cancer tissues in culture, cannot be excluded; furthermore, the basic biological barriers may directly undermine the reprogramming process in cancer cells.

In addition to particular characteristic of pluripotent stem cells, such as their self-renewal and differentiation capabilities, they can repair human tissues after chemotherapy (66). Clinically, after treatment of malignancies with high-dose radiotherapy or chemotherapy, transplanting human stem cells has been generally used to facilitate lifelong hematological recovery (67). The aim of this treatment is to reconstitute the bone marrow under conditions of marrow failure (for example, in aplastic anemia or other blood cell genetic diseases) and to apply human stem cells that differentiate into a desired type of hematopoietic cells in recipients (68). Normally originated iPSCs from patient tissues can theoretically be utilized to regenerate tumor- or treatment- damaged tissues (69). In regenerative medicine approaches, different tissues can be produced using iPSCs. IPSC therapy in regenerative medicine may be valuable in replacing or repairing cancer damaged cell by radiotherapy, chemotherapy, or surgical treatment (70). However, regenerative therapy mediated by human iPSCs needs in vivo extensive studies in iPSC-derived tissues. So far, only a small number of human iPSC-derived cells, such as hepatocytes, have been successfully evaluated in vivo (71, 72).

Table I: Applications of iPSCs in cancer therapy

Origin cells used for generation of iPSC	Reprogramming factors	Cancer type	Type of therapy
Hematopoietic cells (Primary AML cells)	OSKM	Leukemia	investigating the mechanistic basis and clonal properties of human AML, leukemic DNA methylation/gene expression patterns
BRCA1-deleted blood cells	OSKM	breast cancer	Cancer development modeling
Skin cells from RB patient	OSNL	Retinoblastoma	Cancer development modeling
Familial adenomatous polyposis (Colonic organoids)	OSKM	Colon cancer	Modeling of drug Testing in Colorectal Cancer
Hepatic cells	OSKM	Liver cancer	The engraft the liver in a mouse transplantation model
Natural killer cells (BJ1-iPS12, UCBiPS7, and DRiPS16)	OKSM	hematologic and solid malignancies	Anti-tumor therapy (tumor targeting)
human tumor antigen-specific T cells (CD8+ mature T-cells)	OSKM	melanoma	Anti-tumor therapy(tumor targeting)
Dendritic cells (cell lines: 201B7, 253G4, CIRA188Ai-W2, and CB-A11)	OSKM, BMP4	immunological disorders	Cancer immunotherapy such as DC-based vaccines
Juvenile Myelomonocytic Leukemia (JMML)	OKSM	Myelomonocytic Leukemia	Cancer development modeling
glioblastoma (neural lineage)	OSKM, Oct4	glioblastoma	Cancer development modeling

IPSCs and hematopoietic cancer therapy

Developments in nuclear reprogramming of somatic cells lead to new ways of creating pluripotent stem cell lines and represent a significant step in the production of stem cell-based specific therapies (73). Pluripotency reprogramming approach is based on transfer of a somatic cell nucleus into an enucleated oocyte, and applying the expression of specific reprogramming genes in somatic cells (74, 75). These two methods are used to reprogram multiple various cell types, including pancreatic β cells, liver cells, fibroblasts, T and B lymphocyte cells, and neural progenitor cells (76-78). Although many types of cells can be under nuclear reprogramming, the fundamental differences between pluripotent stem cell lines derived from

distinct somatic cell types are largely unclear.

An immune-mediated antitumor effect resulting from allogeneic hematopoietic stem cell (HSC) transplantation might be appropriate for the treatment of some hematological malignancies, such as CML, ALL, AML, Hodgkin and Non-Hodgkin lymphoma, multiple myeloma (MM), sickle cell anemia, thalassemia, and Fanconi anemia (79-81). These HSCs are the most common multipotent stem cells that can differentiate to all blood cell lines through hematopoiesis process (82, 83). After Yamanaka studies in the field of reprogramming, which led invention of iPS technology, many researchers have tried to generate induced hematopoietic stem cells (iHSC) derived from patients with blood malignancy (84, 85). Additionally, these researchers have shown that the specific expression of 13 transcription factors such as OCT4 of OKSM factors can generate HSC. Recently, it has been proven that new genetic engineering tools can be used for producing of iHSCs, including CRISPR-Cas9, meganucleases, zinc-finger nucleases (ZFN), double-strand break (DSB) nucleases, and transcription activator-like effector nucleases (TALEN) (86, 87). Studies have revealed that two transcription factors (HOXA and ERG) act as the core proliferation and differentiation factors in HSC and are inducers of the self-renewal ability, and thus dysregulation of these factors progresses several types of leukemic cells (85). The main differential marker of hematopoietic stem cells is CD34 that help distinguish iHSC in association with other differentiation markers such as CD34, CD38, CD45, CD90, CD105, CD133, and C-kit (stem cell receptor) (83).

1- Reprogrammed T-lymphocytes

Presenting genes encoding chimeric antigen receptors (CARs) or T-cell receptors (TCRs) directed against tumor-related antigens makes HSCs attractive for researches of cancer immunotherapy (88-90). Patient-specific iPSCs can be also potentially helpful in immunotherapy methods (91, 92). The pre-rearranged TCR gene is reserved in T lymphocyte-derived human iPSCs, which can be further differentiated into functionally active T cells (93, 94).

In recent years, various technologies, generally based on reversing immunosuppression, have been expanded by utilizing transferring cytotoxic T lymphocyte (CTL) (95) and transferring TCR gene (96). It is also necessary that they differentiate into double positive cells (CD4/8+), before generating iPSC-derived CTL (iCTL), by IL-2 which is the major cytokine in T-cell differentiation (97, 98). Experimentally tumor antigen-specific T lymphocytes can be generated in vitro by reprogramming designated T cells into iPSCs which are then differentiate back into T lymphocytes for infusion in patients. However, the safety of T cell-derived human iPSCs must be more credible (99, 100).

Since most cancers in a specific tissue include acquired nucleotide mutations, iPSCs resulted from other normal tissues of the same patient hypothetically can be used to regenerate those injured tissues by the tumors themselves or subsequent treatments (101, 102). However, applications the iPSC-derived tissues in cancer therapy, needs that confiremed by in vivo studies. It has been made clear that human iPSCs derived from T lymphocytes maintain the pre-rearranged T cell receptor (TCR) gene; therefore, these iPSCs can be induced for differentiation into functionally active T cells (93, 94). In 2018, Chao et al., described the generation of AML-iPSCs from two patients, with more emphasis on the notion that reprogramming of primary leukemias was not an unimportant assignment (103). Nevertheless, natural or technical reprogramming difficulties are significant barriers to reprogramming of primary

acute leukemic cells that need further investigation.

The differences in reprogramming pre-rearranged cells in AML and B-ALL propose that epigenetic reprogramming may be dependent on the specific genetic alterations and leukemia tumor subtype (104-106). Recent studies have shown that dendritic cells derived from human iPSCs can be completely functional for the assessment of hematopoietic diseases (107-109). However, these cells still need in vivo testing to confirm the effectiveness and safety of their application.

2- Induced NK Cells

Human natural killer (NK) cells are considered as an significant part of the innate immune system by producing significant cytokines for killing virally infected and/or malignant cells (110). While antitumor T-cell immune responses involve a diagnostic initiating phase and their responses are restricted to human leukocyte antigen (HLA), mature NK cells effectively kill malignant cells without any previous exposure (111). In addition, NK cells are the link between innate and adaptive immune systems that can increase and accelerate immunologic responses using their memory ability. At present, these cells are extracted by leukapheresis from the blood circulation and then separated from other cells via magnetic beads coated with anti-CD56. Both NK cell - and T cell -based adoptive immunotherapies have been used to treat patients with tumor malignancies. In recent studies, $CD34^+$ hematopoietic progenitor cells derived from umbilical cord blood (UCB) are also being used as a basis for the generation of many numbers of allogeneic NK cells (112). Some research groups have defined various protocols for the production of NK cells from $CD34^+$ cells using culture of stromal cell lines and a combination of cytokines that progress the expansion of NK cells (113-115). Furthermore, other researchers have been able to produce large numbers of UCB $CD34^+$ cells-derived NK-cells for adoptive immunotherapy in large-scale bioreactors, and for the use in future clinical cancer trials (116, 117). According to the studies on induced NK cells based on iPSC (iNK cells), reprogrammed NK cell can supply the desired volume of cell product to be used in leukemia therapy panel without any immunological response.

Conclusion

Induced pluripotent stem cells provide an alternative approach to human embryonic stem cells (hESCs) without any ethical concerns and with universal usage. Since they are obtained from dedifferentiation of adult cells and not from embryo, iPSCs overcome the ethical hurdles of embryonic stem cells. There are still significant obstacles that need to be considered. Despite the attractive research on iPSC-derived cell transplantation in the grafts, there is still a potential risk of tumor formation. It is possible to generate patient specific iPSC from somatic tissues, which can contribute to drug development, disease modeling, and autologous stem cell therapy. Stem cells migrate to solid tumors and facilitate site-specific anti-tumor drug delivery. It is clear that induced hematopoietic stem cells -derived immune cells provide a genetic manageable system to study human immune cell function and progress. In addition, these immune cells provide a main source of lymphocytes and may open new doors for cancer therapy. Finally, these studies have demonstrated that cell reprogramming in primary tumor cells is more difficult, and that further technological development is needed to be able to generate reliable iPSC models of cancers.

Conflicts of interest

There are no conflicts of interest.

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