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Concise Reviews: Induced Pluripotent Stem Cells as New Model Systems in Oncology

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Key Words. Induced pluripotent stem cells • Cancer • Genetic predisposition • Clonal evolution

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Received March 20, 2015; accepted for publication May 31, 2015; first published online in STEM CELLS EXPRESS Month 00, 2015.

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1066-5099/2014/$30.00/0

http://dx.doi.org/10.1002/stem.2099

ABSTRACT

The demonstration that pluripotent stem cells could be generated by somatic cell reprogramming led to wonder if these so-called induced pluripotent stem (iPS) cells would extend our investigation capabilities in the cancer research field. The first iPS cells derived from cancer cells have now revealed the benefits and potential pitfalls of this new model. iPS cells appear to be an innovative approach to decipher the steps of cell transformation as well as to screen the activity and toxicity of anticancer drugs. A better understanding of the impact of reprogramming on cancer cell-specific features as well as improvements in culture conditions to integrate the role of the microenvironment in their behavior may strengthen the epistemic interest of iPS cells as model systems in oncology. STEM CELLS *2015; 00:000–000*

SIGNIFICANCE STATEMENT

The ability to reprogram any type of differentiated cell into a pluripotent cell, the so-called induced pluripotent stem (iPS) cells, has opened new perspectives in cancer research. This review discusses the already tested opportunities that using cancer cell-derived iPS clones has created and proposes additional applications, especially when other models have failed. More specifically, we discuss the possibility to capture various stages of the disease, including premalignant steps and disease predisposition, and to model cancer stem cells. iPS cells could be also appropriate models to test the activity of drugs and to detect some toxic effects.

INTRODUCTION

The limits inherent to every model used in cancer research restrict the scope of potential investigations. Induced pluripotent stem (iPS) cells, which are pluripotent stem cells generated by inducing somatic cell dedifferentiation, have recently been tested as new models in cancer research. We are just at the beginning of understanding how this model could extend our investigation capabilities in the cancer field.

The technology allowing the generation of iPS cells was first established by Kazutoshi Takahashi and Shinya Yamanaka in mouse fibroblasts [1], and was subsequently applied to human cells [2]. Dedifferentiation and reprogramming were made possible by expressing in differentiated cells, four transcription factors that are usually expressed in pluripotent embryonic stem cells (ESCs), namely Oct-4, Sox2, Klf4, and C-Myc. Following retrovirus-mediated transduction of the genes encoding these four transcription factors, some fibroblasts in culture switched to a pluripotent undifferentiated state. By escaping the fierce of controversy provoked by the use of human ESC, the ability to generate iPS cells was considered as a breakthrough offering new potential opportunities for regenerative medicine, genetic disorder modeling, drug discovery, and drug safety testing.

Since 2009, the reprogramming technology has been successfully applied to cancer cells. Cancer iPS clones were generated from melanoma, chronic myeloid leukemia (CML), gastrointestinal carcinoma, squamous cell carcinoma, and several other cancer-derived cell lines. iPS clones were also obtained by reprogramming CML, juvenile myelomonocytic leukemia, myeloproliferative neoplasm, prostate cancer, and pancreatic ductal adenocarcinoma primary cells. These studies provided the proof of principle that cancer cell-derived iPS clones may be useful models for oncology research.

THE DEMONSTRATED ADVANTAGE OF USING IPS CELLS AS CANCER MODELS

iPS clones provide unique information on the consequences of germline mutations that

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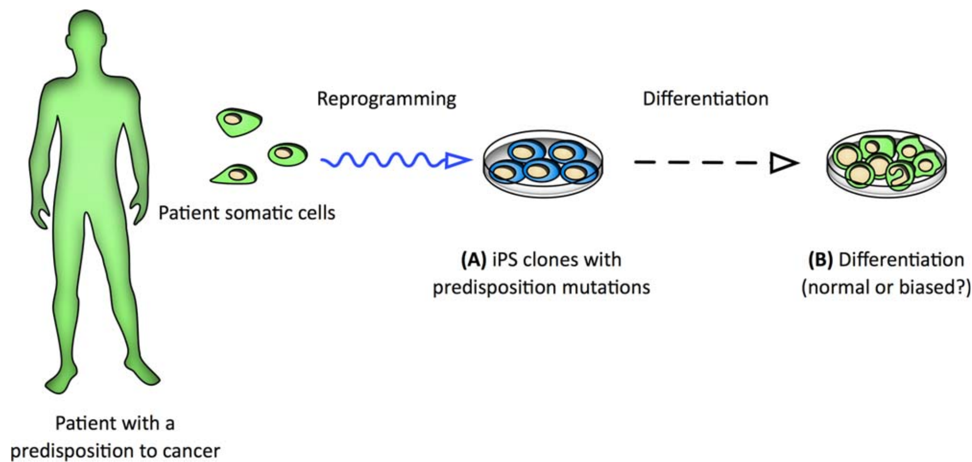

Figure 1. Generating iPS clones to study cancer predisposition. Adult somatic cells from patients carrying a genetic predisposition can be reprogrammed into iPS cells. A phenotype will be observed at the iPS cells stage (e.g., abnormal proliferation rate and accumulation of genomic damages) **(A)** or after inducing iPS cells in vitro differentiation into specialized cells **(B)**. Abbreviation: iPS, induced pluripotent stem.

predispose to malignancies (Fig. 1). For example, FPD/AML (familial platelet disorder with a predisposition to acute myeloid leukemia) is an autosomal dominant disease caused by germline heterozygous mutations in *RUNX1* gene. The attempts to create FPD/AML animal models have been disappointing. Heterozygous *Runx1* mutation leading to haploinsufficiency does not induce any disease. The complete knockout of *Runx1* gene in mice is embryonic lethal whereas knockout induced in adult animals generates a thrombocytopenia and, in some cases, a myeloid malignancy. iPS clones have been generated from FPD patient cells. These clones display the defect in megakaryocytic differentiation that characterizes FPD and the correction of *RUNX1* mutation in these clones, through either gene editing [3], or overexpression of wild-type *RUNX1* [4], normalizes megakaryopoiesis. These results enforce the demonstration that *RUNX1* mutations and *RUNX1* dosage are responsible for the defect in megakaryopoiesis observed in FPD. Analysis of FPD/AML iPS clones demonstrated also, and for the first time, the crucial role of *RUNX1* in regulating the first wave of human primitive hematopoiesis [5]. This predisposition to leukemia appeared to involve an increased proliferation of myeloid progenitors and a genomic instability, which were inversely related to *RUNX1* expression level. The interest of iPS clones to explore the consequences of transcription factor gene dosage was recently validated in other settings [6].

FPD/AML-derived iPS clones are now used in an attempt to decipher initial steps of transformation. The proof-of-concept that iPS clones could be helpful to identify the initial steps of cell transformation has been obtained using iPS cells derived from patients with a Fanconi anemia, another genetic disease that predisposes to malignancies and cannot be fully recapitulated in mice [7]. Fanconi anemia-derived iPS cells demonstrate defects in their ability to generate hematopoietic, mesenchymal, and neural cells. Methylation changes were identified in the generated neural stem cells, leading to the deregulation of tumor-promoting and tumor-suppressor gene expression. Such deregulation may account for the formation of medulloblastomas [8].

Constitutive trisomy 21 (T21) also affects diverse tissues including the hematopoietic tissue. Approximately 10% of T21 newborns exhibit a clonal preleukemia, referred to as a transient myeloproliferative disease, which is always associated with an acquired mutation in *GATA-1* exon 2 leading to a short form of this transcription factor (GATA-1s). This transient situation evolves into acute megakaryoblastic leukemia in 30% of cases through acquisition of additional mutations. The impact of an additional chromosome 21 on embryonic and fetal hematopoiesis is difficult to determine in animal models. Interestingly, differentiation of iPS clones generated from T21 patients demonstrated the abnormal differentiation of iPS-derived blood progenitors [9], providing models for further mechanistic analyses. This approach, together with fetal liver analyses, demonstrated that trisomy 21 alters fetal but not adult hematopoiesis. Accordingly, the transient myeloproliferative disease observed in some T21 newborns disappears a few months after birth when hematopoiesis switches from the fetal liver to the bone marrow.

Finally, iPS clones were established from patients carrying a constitutive mutation in *BRCA1* or *BRCA2* tumor-suppressor genes. BRCA1 and BRCA2 are key players in DNA repair, gene transcription, cell-cycle regulation, polyadenylation of messenger RNA, and ubiquitinylation. Mice have been genetically engineered to model BRCA1 and BRCA2 deficiencies. These animal models demonstrated some limits, for example, mutation in a single *BRCA1* allele leads to genomic instability in human cells but not in mouse cells [10]. Analysis of iPS clones derived from patients carrying a *BRCA1* mutation provided new insights in their biological effects, such as the identification of an increase in the expression level and activity of protein kinase-C-theta. Interestingly, the reprogramming-associated de novo mutation rate was not higher in *BRCA1* mutants than in control iPS cells. These clones were used to screen therapeutic compounds that could prevent the development of tumors in genetically predisposed patients [11].

Cancer-derived iPS clones have also been used to model early stages of cancers in the absence of identified predisposition. Generation of cancer cell lines as well as serial transplantation of cancer cells in the mouse usually require features that are characteristic of the most aggressive cancer types, such as a high proliferative index of disease

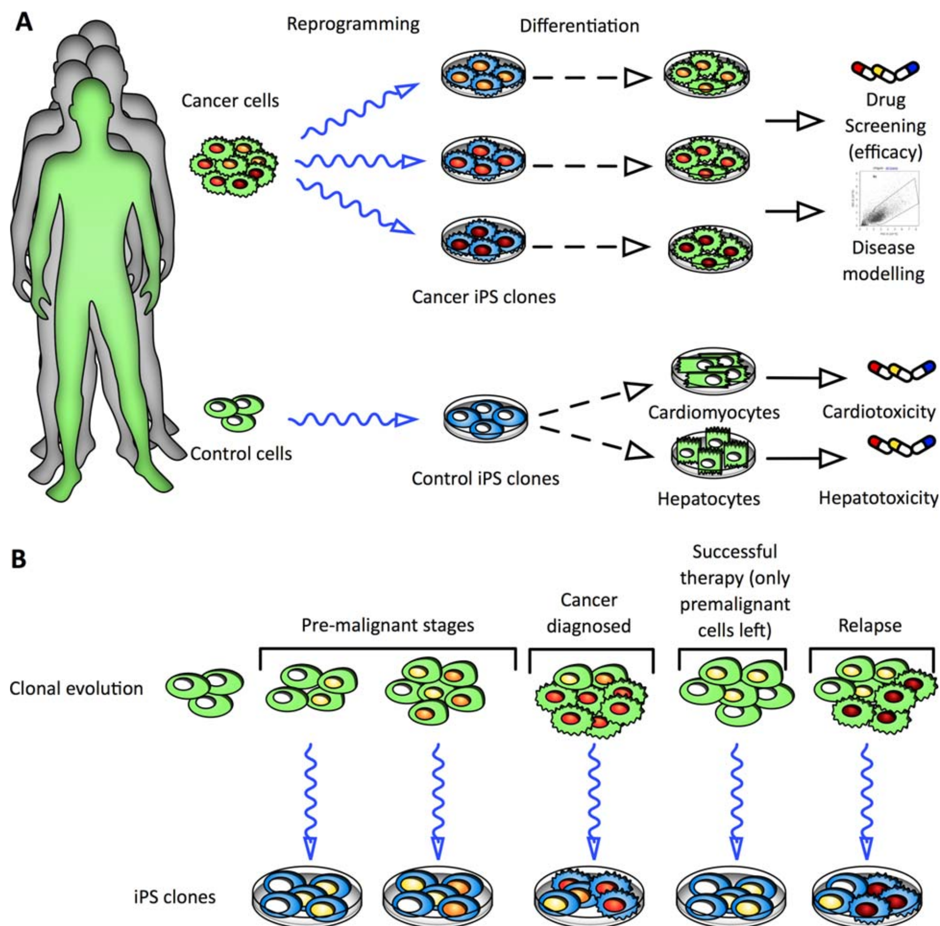

Figure 2. Generating iPS clones to study drug efficacy and toxicity, and cancer progression. **(A):** iPS clones with different genetic background could be established from a tumor sample and used to explore the heterogeneity of tumor cell biology and sensitivity to drugs. iPS clones generated from normal cells (control) of the same patient will be differentiated into specialized cell types to detect tissue-specific toxicities of the studied drugs. **(B):** iPS clones could be used to model and explore various stages of disease progression, including premalignant stages, malignant stages, therapeutic response, and relapse. Abbreviation: iPS, induced pluripotent stem.

propagating cells. Modeling of early phases of cancer progression is a much more difficult task. iPS clones could be derived from cancer cells at any stage, including the earliest. For example, a pluripotent iPS cell lines established by reprogramming human pancreatic ductal adenocarcinoma (PDAC) cells were observed to generate pancreatic intraepithelial neoplasia precursors to PDAC when injected to immunocompromised mice. The generated tumor subsequently progressed to an invasive stage [12]. This observation suggested that iPS cell generation could be used to identify early stages of cancers, providing new insights into disease progression mechanisms.

Last but not least, cancer-derived iPS clones were used to explore the tumor cell response to anticancer drugs (Fig. 2A). An exciting observation was made in iPS clones established from CML [13–15]: the *BCR-ABL* fusion gene, which is a molecular signature of CML, encodes a tyrosine kinase whose chemical inhibition with tyrosine kinase inhibitors (TKI), such as Imatinib, has dramatically improved the disease outcome. It was established however that TKI do not eradicate CML stem cells. Interestingly, iPS cells derived from patients responding to Imatinib are resistant to the drug, and inducing their differentiation into hematopoietic progenitors restores their sensitivity. Thus, iPS cells recapitulate a characteristic feature of the disease, providing a model to explore the mechanisms by which leukemia propagating cells resist to TKI. Another disease in which malignant cell-derived iPS clones were tested for their ability to model the diseased cell response to various drugs is juvenile myelomonocytic leukemia (JMML), an aggressive myeloproliferative neoplasm/myelodysplastic syndrome in which myeloid progenitors are hypersensitive to the granulomonocyte colony-stimulating factor. This characteristic feature was conserved in hematopoietic cells generated by differentiation of JMML-derived iPS cells, allowing to examine the activity of kinase inhibitors on these differentiated cells [16]. We and others used a similar approach to explore the activity of Janus-activated kinase 2 (JAK2), phosphatidylinositol-3-kinase, and Heat Shock Protein 90 inhibitors on hematopoietic cells generated by inducing the differentiation of JAK2^{V617F} myeloproliferative neoplasm-derived iPS cells [17, 18].

Altogether, these models illustrate the ability of cancer-derived iPS cells to model differentiation bias induced by genetic alterations that predispose or contribute to cell transformation and to explore cancer cell response to various anticancer drugs. Their interest may further increase with the development of gene editing techniques, allowing manipulation of these cellular models.

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The Potential Benefits of iPS Cells for Future Research

A first issue will be to determine whether cancer-derived iPS cells could bring an input to the cancer stem cell (CSC) controversy. CSCs were proposed to be the driving force of cancer development and progression, and the cause of some therapeutic failures [19, 20]. As they often represent a tiny fraction of cancer cells, the possibility to generate a great number of them using iPS technology would be of great interest [21]. However, the existence and the role of CSCs remain controversial issues. First, some cancers may not be hierarchically organized [22], and the challenge to model CSCs from iPS clones in these cancers has limited relevance. Second, in hierarchically organized tumors, some cancer non-stem cells could dedifferentiate and acquire the properties of CSCs [23, 24]. In these latter cases, iPS cells may be useful to explore the dedifferentiation of somatic cells following an oncogenic insult [25, 26]. More generally, it might be informative to identify the features that allow a minor fraction of transduced cells to achieve reprogramming.

The challenge will be to determine the right stage of iPS cell differentiation that reproduces CSC features. The fact that, before any induction of differentiation, CML-derived iPS clones resist to imatinib similarly to CML propagating cells [14, 15] does not mean that they are CML stem cells, nor that they resist through mechanisms that are similar to those developed by primary leukemic stem cells. By reprogramming a mammary epithelial cell line into iPS cells, then inducing their differentiation, Nishi et al. obtained so-called CSC-like cells that were used to screen compounds that selectively target CSCs such as salinomycin [27] and withaferin [28]. Several other studies claimed they had reprogrammed tumor cells [21, 29] and even normal cells [30] into CSC-like cells. While all these studies remain insufficient to demonstrate the ability to truly model CSCs, the research topic seems worthwhile to be explored. If the generation and subsequent differentiation of cancer iPS clones established from cancer cells can model the disease, then one can legitimately expect that iPS clones also model, and expand, the CSCs.

Another field in which iPS cell technology may open avenues is the analysis of biological steps that forerun the clinical development of cancers (Fig. 2). In these recently described premalignant clones, somatic mutations in one or several genes increase the rates of cancer development. For example, some monozygous twins were observed to develop an acute leukemia from a common preleukemic clone established in utero [31]. Mel Greaves has recently proposed that, given the rate of spontaneous mutations, the rate of cell divisions, and our life duration, we may all grow premalignant cells [32]. Increasing evidence indicate that hematopoiesis can become clonal with ageing, in the absence of any overt disease [33–36]. There is evidence that hematopoietic stem cells lose a part of their capacities during aging, due to both intrinsic and extrinsic changes. For example, mutations affecting *TET2* and *DNMT3A* genes could increase stem cell fitness while being the ground for additional oncogenic mutations leading to malignancies [37, 38]. Importantly, these preleukemic (initiating) clones could resist to treatment and regenerate malignant clones over time through occurrence of new mutations [39].

Since all the clones do not evolve into a malignant disease, the challenge is to identify and to target premalignant lesions with great propensity to evolve further into life-threatening cancers. We lack models to study the biological properties of premalignant clones and how the mutated cell ultimately transforms into a malignant one. Due to their relatively short life span, mouse models are poorly appropriate for studying predisposition to cancer, and it is likely that it will be the same to explore stepwise progression to malignancy. Cell lines are generated from fully malignant cells, not from premalignant stages. Therefore, establishing iPS clones from premalignant cells that accumulate with age might offer an unprecedented opportunity to explore their biological features and to better identify the steps that precede cancer-initiating events (Fig. 2B), that is, these models may distinguish the consequences of increased proliferation from those of genetic instability and replicative stress.

We have shown that cancer-derived iPS clones could be used to screen anticancer drugs. The causes of failure in the development of an anticancer drug, either lack of efficacy or toxicity to healthy tissues, have to be identified as early as possible [40]. In addition to being a potential alternative to animal models, cancer-derived iPS cells could be closer to clinical situations than many other cancer models as they capture the complex genetic background of patient tumors. These "diseases-in-a-dish" may be used early in the preclinical drug development process [41]. Importantly, cancer cell reprogramming could capture a part of tumor heterogeneity, underlining the interest of testing several independent clones generated from a same tumor. When needed, RNA interference technology and gene editing can reproduce genetic alterations that have not been captured by tumor cell reprogramming. Then, the limitless expansion of cancer iPS cells allows to simultaneously test multiple compounds at multiple time points, and to compare cancer-derived iPS cells to iPS cells derived from normal cells with the same genetic background. Finally, high throughput screens could compare cancer cell-derived iPS clones established from a number of patients to determine whether a drug efficacy depends on the tumor cell genetic background, the results of these screens being used to guide the development of precision medicine [42, 43].

As their differentiation into specific cell types provides opportunities to screen the toxic effects of the drugs on normal tissues, iPS clones established from healthy donor cells will also facilitate anticancer drug development. For example, cardiomyocytes obtained by differentiation of iPS cells, which faithfully recapitulate adult human ventricular cardiomyocyte properties, could be used to anticipate increase in QT interval arrhythmias, and other cardiac side effects of a developed drug [44]. Human iPS cells can also be differentiated into functional hepatocytes that are promising tools to screen for drug hepatotoxicity [45] (Fig. 2A). Early detection of a toxic effect may limit the cost of drug development and provide opportunities to modify chemically the candidate drug in order to suppress its toxic effects without eliminating its therapeutic properties.

How to Improve the Epistemic Interest of Cancer-Derived iPS Clones?

As every other cancer model, cancer iPS clones have intrinsic limitations inherent to the model. The first one is that probably all the tumor cells cannot be reprogrammed. For

example, TET2 is a 5-methylcytosine dioxygenase that could be involved in complete reprogramming [46]. Homozygous loss of function mutations in *TET2* gene, which are frequently observed in hematopoietic malignancies, is therefore suspected to prevent leukemic cell reprogramming.

The second limitation is that cancer cell reprogramming may alter their epigenetic landscape. These modifications could potentially eliminate some of the characteristic features of the cancer cell phenotype, creating a distance between the cancer iPS model and the original cancer cell. According to this hypothesis, it was even proposed that reprogramming could be used therapeutically to revert the cancer phenotype [47, 48], although it will not eliminate the gene mutations that characterize the transformed cells. iPS cell technology also faces some extrinsic limitations that may be overcome by improving the culture conditions used to maintain or expand cancer iPS clones, to induce their differentiation, and to explore their ability to generate tumors in immune-compromised animals. For example, using endothelial cells that overexpress Notch ligands jagged-1 and delta-like ligand-4 improves the formation of hematopoietic multipotent progenitor from pluripotent stem cells and markedly increases the engraftment of the iPS cells in NOD/SCID/IL-2 receptor ɣ chain-null mice [49].

Last but not least, as physiologically integrated and functionally autonomous entities maintained in culture, cancer iPS clones fail to model the role of the cancer cell interactions with surrounding tissues in their development. Improvements of in vitro culture conditions to partially model the tumor cell environment would strengthen the epistemic interest of cancer iPS cells. For example, iPS cells could be used to form organoids [50] and organ-on-a-chip, which are a multichannel three-dimensional microfluidic cell culture chip aiming at mimicking entire organs such as lung, heart, kidney, artery, or gut, which are presented as “promising substitutes for animal testing” [51]. However, organ-on-a-chip is not patient-on-a-chip. Cancer iPS cells cannot answer all the questions raised by tumor arising, development, and response to therapy, neither can any other model, but they may provide some new clues that no other model offers.

Acknowledgments

E.S. team is supported by grants from the French National Cancer Institute and labeled by the Ligue Nationale Contre le Cancer. W.V. team is labeled by the Ligue Nationale contre le Cancer. L.L. is supported by SOCRATE program, and platforms are supported by Molecular Medicine in Oncology (MMO) and Paris Alliance for Cancer Research Initiative (PACRI) programs.

Author Contributions

All authors equally contributed to the manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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