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Induced pluripotent stem cell modeling of malignant hematopoiesis

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The ability to epigenetically reprogram differentiated somatic cells to pluripotency resulting in the discovery of induced pluripotent stem cells (iPSCs), has unlocked fundamental biologic insights into numerous genetic diseases. These insights have resulted from the key property of iPSCs to differentiate into all cell lineages in an unlimited manner while maintaining the genetic identity of the originating cell. iPSCs have been utilized to investigate both monogenic and complex genetic disorders spanning hereditary and acquired diseases. Recently, iPSCs have been utilized to model human cancer, with a specific focus on modeling conditions of malignant hematopoiesis. In addition to serving as a genetic disease model in cancer, iPSCs can also be used as a tool to address a key question in interrogating the interaction between the cancer epigenome −genome. Specifically, how does reprogramming the epigenome affect cancer phenotype and specifically malignant hematopoiesis? This review will address this question and highlight the state of the field in generating iPSCs from hematologic malignancies, key biologic insights that can be uniquely generated from cancer-derived iPSCs, and their clinical applications. Last, challenges to expanding the use of iPSC modeling in blood cancers will be discussed.

In 2012, the Nobel Prize in Physiology or Medicine was awarded to Sir John Gurdon and Shinya Yamanaka for the discovery that mature differentiated cells could be reprogrammed to a pluripotent state. By introducing four transcription factors (Sox2, Klf4, Oct3/4, and c-Myc), terminally differentiated cells can be epigenetically reprogrammed to an undifferentiated state [1]. Importantly, this epigenetic process does not alter the underlying genetic code of the parent cell. Therefore, these induced pluripotent stem cells (iPSCs) have significant applications in disease biology, including the ability to create novel disease models of human genetic disease for biologic discovery. Understanding the genetic basis of disease through iPSCs has led to their use as a model for therapeutic drug screening. In addition, the potential unlimited proliferative potential of iPSCs into differentiated cells enables a patient-specific supply of cell types that can be used for regenerative medicine. Although the primary use of iPSCs has been in investigating nonmalignant monogenetic disorders, recently, iPSC technology has been applied to cancer as a tool to better understand the genetic drivers of oncogenesis. Generation of iPSCs from cancer patients has been successfully reported in both solid tumors and hematologic malignancies. This review will describe the current state of patient-derived iPSC generation in malignant hematopoiesis, highlight key biologic insights gained from these iPSC models, discuss clinical and therapeutic applications, and outline the challenges to expanding iPSC technology in blood cancers.

Generation of patient-derived iPSCs from multiple hematologic malignancies

Although most iPSC-based models have been focused on diseases caused by a single gene, in recent years, iPSC technology has been utilized to generate disease models in human malignancies, which comprise a more complex genetic background. Modeling somatic mutations that arise in cancer and the relative contributions of multiple mutations to cancer initiation is a key area of ongoing research and can be successfully modeled by patient-derived iPSCs. Although iPSCs have been successfully generated from a small number of solid tumors, the generation of iPSCs from hematologic

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malignancies has been more robust. In blood cancers, iPSCs have been generated from both cancer cell lines and, more importantly, from primary patient samples. Successful generation of blood-cancer-derived iPSCs has been performed in a diverse set of human blood cancers including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS), polycythemia vera (PV), primary myelofibrosis (PMF), and juvenile myelomonocytic leukemia (JMML; Table 1). Studies included in Table 1 demonstrated key criteria for successful generation of iPSCs derived from blood cancers, including: (1) the formation of functional iPSCs as assessed by in vitro and in vivo characteristics, (2) the retention of underlying cancer somatic mutations upon reprogramming, and (3) the characterization of disease phenotype in blood-cancer-derived iPSCs. Reprogramming methods consisted of transduction of the classic Yamanaka factors (Sox2, Klf4, Oct3/4, and c-Myc). However, methods of delivery were varied and included retrovirus, lentivirus, the nonintegrating Sendai virus, and episomal vectors. In general, these studies demonstrate that cancer-derived iPSCs possess phenotypes of the original patient cancer. In myeloid cancers, these include aberrant in vitro myeloid-restricted differentiation potential, cytokine-independent cell proliferation, and gene expression profiles similar to the original patient. Until recently, in vivo recapitulation of disease was not reported and was a key barrier to the expanded utilization of iPSCs in modeling in blood cancers (Table 1). However, two recent reports have demonstrated the ability of AML/MDS-derived iPSCs to recapitulate leukemia in vivo when transplanted into immune-deficient mice [2,3].

Interestingly, published reports detail the successful generation of iPSCs mainly from human myeloid malignancies rather than lymphoid malignancies. Part of the reason for the dearth of iPSC generation in lymphoid malignancies is likely due to the lack of large amounts of human patient tumor tissue available for such cancers like lymphoma, as opposed to the readily available cancer cells in the peripheral blood in most myeloid malignancies. However, pluripotent reprogramming has been attempted in some lymphoid malignancies (mainly acute lymphoblastic leukemia [ALL]) [4], which has not been successfully reported to date. Challenges to generation of cancer-derived iPSCs are discussed in Challenges to iPSC Reprogramming of Hematologic Malignancies.

The majority of these reports describe successful iPSC generation in blood cancers that have a single dominant driver mutation. For example, multiple studies have demonstrated the generation of patient-derived iPSCs in myeloproliferative neoplasms driven by a single driver mutation (i.e., BCR-ABL in CML and JAK2 in PV and PMF). However, several recent reports have described the generation of iPSCs in myeloid malignancies with multiple genetic abnormalities, particularly in AML and MDS [2,3,5]. The robustness and scalability in generating patient-derived iPSCs in blood cancers with complex mutational and chromosomal aberrations remains to be explored.

Biologic insights of patient-derived iPSCs in hematologic malignancies

Epigenetic dysregulation is an established feature in many hematologic malignancies and plays a significant role in disease pathogenesis. These epigenetic abnormalities include aberrant DNA methylation, histone modifications, and chromatin accessibility. Although genetic mutations serve as drivers of oncogenesis, epigenetic dysregulation may independently contribute to disease development, a concept broadly proposed in cancer referred to as epigenetic stochasticity [6]. This model proposes that cancer mutations act within the context of an epigenetic setting conducive to oncogenesis, often with epigenetic dysregulation establishing this context. Although there is clearly a dynamic interplay between genetic mutations and epigenetic modifications, this relationship is poorly understood. Cancer-derived iPSCs can serve as a key tool to understand the contributions of genetic versus epigenetic events in oncogenesis. Through the introduction of a defined set of transcription factors, cancer cells can be epigenetically reprogrammed to a pluripotent state while leaving underlying genetic abnormalities intact. The ability to globally reverse epigenetic alterations separate from genetic abnormalities through iPSC derivation enables the understanding of whether oncogenic driver mutations are dependent on a specific epigenetic environment. Several studies have investigated this interplay in hematologic malignancies, specifically within AML and CML. First, human AML patient cells containing multiple leukemic driver mutations (mixed-lineage leukemia [MLL] rearrangements, KRAS mutations, and others) were reprogrammed to pluripotency with the underlying driver genetic mutations intact [3]. The leukemogenic potential of these AML iPSCs was then investigated in the context of differentiation down specific cell lineages. Not surprisingly, when AML iPSCs were differentiated down the hematopoietic lineage, these cells reacquired leukemic properties including serial replating potential and in vivo engraftment of leukemia when transplanted into mice. In contrast, when AML iPSCs were differentiated into nonhematopoietic (neural and cardiac) lineages, these iPSCs displayed no leukemic or oncogenic properties. Therefore, cancer formation of AML iPSCs was dependent on a hematopoietic cell lineage context. To understand what epigenetic changes could drive leukemogenesis in this system, DNA methylation and its impact on gene expression was investigated. Interestingly, aberrant hypomethylation of MLL fusion target genes (the

Table 1. iPSC generation in human hematologic malignancies

Cancer type	Reprogramming method	Cell type reprogrammed	Cancer-derived iPSCs	Disease functional findings		Reference
				In vitro	In vivo	
AML	Sendai virus	AML patient (MLL rearranged) peripheral blood cells	2	Colony-forming assay showing myeloid-restricted differentiation compared with normal; mutational analysis showing multiple patient mutations retained in iPSCs; DNA methylation, and gene expression analysis showing similarity of differentiated iPSCs to patient cells	Leukemia formation when iPSC-derived hematopoietic cells were engrafted in NSG mice; Leukemic engraftment of secondary transplants	[3]
MDS/AML	Lentivirus	Low-risk MDS, high-risk MDS, secondary AML patient bone marrow or peripheral blood cells	4	Colony-forming assay showing myeloid-restricted differentiation compared with normal; Gene expression analysis of low- to high-risk MDS/AML iPSCs recapitulating disease progression signatures of MDS to AML patients	Leukemia formation when iPSC-derived hematopoietic cells were engrafted in NSG mice from AML (but not MDS) iPSCs	[2]
AML	Sendai virus	AML patient peripheral blood cells	1	Mutational analysis showing ASXL1 and CALR mutations retained in iPSCs	None	[19]
AML	Sendai virus, lentivirus	MLL-rearranged AML patient bone marrow and peripheral blood cells	1	Colony-forming assay showing differentiation immaturity similar to patient AML cells	None	[14]
CML	Retrovirus	CML cell lines (K562, KBM7) and CML patient bone marrow cells	3	Mutational analysis showing BCR-ABL translocation retained in iPSCs; OP9 stromal coculture differentiation assay restores myeloid and erythroid differentiation of CML iPSCs; DNA methylation analysis showing erasure of leukemia-specific patterns upon iPSC reprogramming of CML cells	No leukemic engraftment seen when iPSC-derived hematopoietic cells were transplanted into NSG mice	[9]
CML	Retrovirus	CML cell line (KBM7)	1	Mutational analysis showing BCR-ABL translocation retained in iPSCs; Limited analysis of hematopoietic differentiation	None	[20]
MDS	Lentivirus	Del(7q)$^{-}$ MDS patient hematopoietic cells	2	Mutational analysis showing (del(7q)) retained in iPSCs; Embryoid-body-based differentiation assay showing reduced hematopoietic differentiation potential and clonogenic capacity compared with normal	None	[5]
MPNs such as PV and PMF	Retrovirus	CD34^{+} MPN patient peripheral blood cells	2	Mutational analysis showing JAK2 mutation retained in iPSCs; Increased erythroid colony formation and proliferation of iPSCs compared with normal; Gene expression similar to primary patient cells	None	[21]
PV	Episomal vector	JAK2^{+} PV patient peripheral blood cells	1	Mutational analysis showing BCR-ABL translocation retained in iPSCs;	None	[13]

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Table 1 (*Continued*)

Cancer type	Reprogramming method	Cell type reprogrammed	Cancer-derived iPSCs	Disease functional findings		Reference
				In vitro	In vivo	
				Erythroid differentiation showing abnormal erythropoiesis (EPO independent) compared with normal;		
				Inhibition of erythropoiesis by JAK2 inhibitors		
MF	Retrovirus	Primary and secondary myelofibrosis patient bone marrow cells	2	Mutational analysis showing JAK2 and del(13) mutations retained in iPSCs;	None	[18]
				Increased IL-8 expression in differentiated megakaryocytes compared with normal		
JMML	Lentivirus	JMML patient (PTPN11 mutant) bone marrow and blood cells	2	Mutational analysis showing PTPN11 mutation retained in iPSCs;	None	[22]
				Monolayer differentiation cultures showing increased myeloid proliferation and constitutive granulocyte-macrophage colony-stimulating factor activation compared with normal		

EPO=Erythropoietin; *MF*=myelofibrosis; *MPN*=myoproliferative neoplasm; *NSG*=NOD-scid-IL2 receptor gamma null.

major oncogenic mutation in these AML samples) leading to their increased expression was observed when AML iPSCs were differentiated into hematopoietic cells. These aggregate results were also confirmed in a mouse model overexpressing the MLL-AF9 oncogene that was inducibly reprogrammed to pluripotency [7]. Upon addition of doxycycline, mouse leukemia cells were converted into iPSCs in vivo with RNA-sequencing analysis, demonstrating reversible global gene expression patterns. Similar to findings by Chao et al. [3], these iPSCs resulted in leukemia formation in vivo that displayed a similar MLL target gene activation signature. Findings from Chao et al. [3] and Liu et al. [7] suggest that certain genetic mutations in AML iPSCs are driven by an epigenetic context, including differentiation status of the cell.

Do other cancer types behave similarly to AML with regard to epigenetic reprogramming? Several studies suggest that the reacquisition of oncogenesis upon epigenetic reprogramming is different in various cancer types. In glioblastomas, pluripotent reprogramming led to resetting of cancer-specific methylation marks [8]. However, differentiated neural cells from glioblastoma iPSCs retained malignant potential, implying that, similar to the leukemia studies we described, oncogenic properties are dependent on epigenetic context. In contrast, iPSCs generated from CML led to reversal of leukemia-specific DNA methylation marks and even normal hematopoietic differentiation despite the retained presence of the BCR-ABL oncogene [9].

These studies suggest that oncogenic driver mutations act in a context-dependent manner, specifically in relation to the differentiation state of the cell. However, this finding is likely dependent on tumor type. Nevertheless, this observation has potential therapeutic implications because the efficacy of therapeutic targeting of genetic mutations may be dependent on the state of cell differentiation. For example, in MLL-rearranged AML, DOT1L inhibition can therapeutically target the MLL oncogene program and has been investigated in clinical trials in MLL-rearranged AML patients. DOT1L inhibition of AML iPSCs differentiated to hematopoietic cells eliminated leukemic disease [3]. In contrast, DOT1L inhibition had no impact on undifferentiated AML iPSCs. This finding argues that therapeutic targeting of genetic abnormalities may be affected by the differentiation state of the cell.

Functional interrogation of genetic and chromosomal abnormalities with iPSCs through genome editing

With the use of genome editing tools to enable gene modification, iPSCs can also be used as a platform to understand the role of specific somatic mutations and their impact on cancer phenotype. Through gene correction methods such as CRISPR, genetic mutations can be

corrected to wild-type in cancer-derived iPSCs with investigation of the functional consequences. Proof of concept of this application was explored in elucidating the functional consequence of loss of chromosome 7q [del(7q)] in MDS patients, which yielded several observations [5]. First, iPSCs were generated from MDS patients that included both del(7q)$^-$ and isogenic karyotypically normal lines. Second, del(7q)$^-$ MDS iPSCs exhibited reduced hematopoietic potential (assessed by CD34 and CD45 expression) and decreased clonogenic capacity (by colony-forming assays) compared with isogenic lines. Third, in one MDS iPSC clone, spontaneous acquisition of an additional chromosome 7 led to resolution of haploinsufficiency, which resulted in the "rescue" of hematopoietic differentiation defects. To further confirm this phenomenon, chr7q deletions were introduced into normal iPSC lines to evaluate the functional consequence on hematopoietic differentiation. del(7q)$^-$ engineered iPSC clones demonstrated reduced hematopoietic differentiation potential similar to the MDS iPSC del(7q)$^-$ clones. Last, candidate haploinsufficient genes that mediated del(7q)$^-$ impaired hematopoietic differentiation were investigated through a phenotypic screen of genes differentially expressed in isogenic del(7q)$^-$ and wild-type MDS iPSCs. This screen identified EZH2, a gene commonly mutated in MDS patients, as a mediator of hematopoietic defects caused by del(7q). This study serves as proof of concept that IPSCs in combination with genome engineering can be utilized to functionally characterize disease-associated chromosomal abnormalities and other genetic alterations in malignant hematopoiesis.

Clinical applications of patient-derived iPSCs in hematologic malignancies

Several reports have highlighted the use of patient-derived iPSCs as an in vitro drug discovery and characterization platform. These studies have investigated therapeutic approaches in several hematologic malignancies, including AML, MDS, CML, and BCR-ABL$^-$ myoproliferative neoplasms [2,3,10–13]. Three potential uses of iPSCs are valuable for clinical translation: (1) screening novel therapeutic compounds for preclinical efficacy in a patient-specific manner, (2) modeling therapeutic targeting in relationship to the differentiation state of the malignant cell, and (3) therapeutic investigation and targeting of distinct genetic subclones.

First, patient-derived iPSCs can be uniquely utilized as a drug-screening platform, given the ability for these genetically defined iPSCs to continuously proliferate, leading to a sustainable cell supply that is not available with primary patient samples. Indeed, patient-derived iPSCs from CML [9], PV [13], MLL-rearranged AML [3], and JMML [10] enabled characterization of the BCR-ABL, JAK2, and MLL pathways and other kinase inhibitors in their respective disease types. The key advantage to this approach is the ability to conduct drug screens in a genetically defined leukemic iPSC population in parallel with the isogenic wild-type patient iPSC control. Using a CRISPR-based approach introducing specific mutations in MDS-derived iPSCs, Chang et al. [10] evaluated the role of the spliceosome mutation, SRSF2, in MDS therapeutic targeting. Several spliceosome inhibitors, including one in clinical development (www.clinicaltrials.gov identifier NCT00499499), led to selective growth inhibition of SRSF2 mutant, but not wild-type, cells. In addition, patient-derived iPSCs have also been used to identify novel therapeutic targets in a true drug discovery approach. In the same study, an in vitro cell proliferation drug screen against a 2,000-drug library of US Food and Drug Administration–approved agents was conducted in SRSF2 mutant and del(7q) MDS iPSCs along with isogeneic normal cells. This approach identified niflumic acid as a selective inhibitor of del(7q) MDS iPSCs, but not normal counterparts, and was further validated in primary MDS patient samples with chromosome 7 abnormalities. In another approach, CML-derived iPSCs were utilized to identify genes associated with resistance to imatinib, a BCR-ABL tyrosine kinase inhibitor used as a standard treatment for CML [12]. This approach identified ADAM8, a metalloproteinase, as an antigen in tyrosine kinase inhibitor-resistant CML patient cells and as a potential predictor of residual disease or relapse. These approaches serve as examples of identifying novel targets using an iPSC-based drug-screening approach that have the potential for clinical translation.

Second, iPSCs can be used to interrogate relative anticancer activity of therapeutic agents across disease states and differentiation contexts. One of the unique advantages of the iPSC system is the ability to differentiate cells to defined cell types and differentiation states that can then be therapeutically investigated. Two studies have used iPSCs in this fashion. Utilizing iPSCs derived from patients at varying disease stages of MDS, Kotini et al. [2] investigated the therapeutic effects of azacitidine, a hypomethylating agent standardly used in MDS treatment. Interestingly, azacitidine restored differentiation in low-risk MDS with no impact on growth inhibition, whereas inhibition of proliferation was observed in a high-risk MDS clone. In another study, the impact of DOT1L inhibition, an investigational treatment for MLL-rearranged AML, was studied in MLL-rearranged iPSCs [3]. The DOT1L inhibitor EPZ-5676 (investigated in clinical trials) specifically inhibited the growth of MLL-rearranged AML iPSCs differentiated into the hematopoietic lineage with no deleterious effect on hematopoietic cells derived from normal iPSCs. Interestingly, EPZ-5676 exhibited minimal growth inhibition on undifferentiated AML iPSCs compared with hematopoietic cells

differentiated from these AML iPSCs, which suggests that the efficacy of DOT1L inhibition may be dependent on the differentiation state of AML cells and could be a predictor of clinical efficacy [3].

Third, patient-derived iPSC lines can be utilized for investigating the dynamics of disease subclones within a patient. Because iPSC clones are derived from single cells, leukemia-derived iPSC clones can model the characterization and therapeutic targeting of distinct genetic subclones present in an individual patient. This ability is especially unique to iPSCs, given the difficulty in isolating and expanding rare subclones from patient samples. Through generation of multiple AML iPSC lines, two distinct subclones (KRAS mutant and wild-type) were identified in a single patient that shared all other leukemic mutations [3]. These KRAS subclones had differential growth kinetics, dependence on granulocyte-macrophage colony-stimulating factor signaling, in vivo leukemic engraftment potential, and sensitivity to MEK inhibition. Differential sensitivity to MEK inhibition in KRAS-mutant iPSCs compared with wild-type was subsequently validated in a panel of primary AML patient samples. However, what is more striking is that investigation of these KRAS mutant and wild-type subclones enabled the prediction of clonal relapse in this patient. Specifically, AML iPSCs predicted that the KRAS wild-type subclone was refractory to induction chemotherapy and underwent clonal selection and evolution, leading to frank relapse. These findings demonstrate proof of concept that patient-derived iPSCs can capture the clonal heterogeneity of an individual patient case and predict clonal response to therapy and disease evolution.

In summary, patient-derived iPSCs possess unique capabilities for therapeutic translation as a drug-screening platform, evaluating the impact of therapies in different stages of differentiation, and modeling clonal evolution.

Challenges to iPSC reprogramming of hematologic malignancies

Although multiple studies have reported the successful generation of patient-derived iPSCs from a spectrum of hematologic malignancies, this has not been the case for all blood cancer types or all cases of a particular disease. For example, cellular reprogramming was attempted in B-cell ALL (B-ALL) patient samples but was unsuccessful despite the utilization of several different reprogramming vector-based strategies (retroviral, lentiviral, episomal, and Sendai virus) [4]. Seven distinct B-ALL patient samples were tested spanning a variety of genetic and chromosomal abnormalities. In addition, an attempt was made to reprogram 16 AML patient samples, and iPSCs harboring leukemic-specific mutations were successfully generated from only one patient sample [14]. The success of cancer-derived iPSC generation is likely influenced by multiple factors including both underlying patient characteristics and technical variation in reprogramming assays. First, given the observation that reprogramming of malignant cells to iPSCs has not been universally achieved, this in part depends on the specific genetic and chromosomal abnormalities in the patient cells. Prior studies have shown that cells bearing specific mutations (e.g., p53) and complex karyotypic abnormalities can be refractory to pluripotent reprogramming [15,16]. In addition, specific regions of the genome have been shown to be refractory to binding by Oct4, Sox2, and Klf4, which is needed to reprogram cells to pluripotency [17]. Specifically, in the reprogramming of AML, the presence of abnormalities in the MLL gene may positively affect the ability to generate iPSCs. Although few studies describe the successful generation of iPSCs from AML patient samples, those that do have described successful reprogramming in MLL-rearranged AML samples [3,14]. This success may be dependent on the specific cancer type because MLL-rearranged B-ALL samples were unable to be reprogrammed [4]. Further investigation on the biologic role of MLL rearrangements in cellular reprogramming is needed. It should be noted that iPSCs have been successfully generated from AML patients without MLL rearrangements, including an AML patient with complex karyotype (who had a MLL3 mutation of unknown significance but no MLL rearrangement; personal communication) [2]. In addition, we have also generated iPSCs from an AML patient with normal karyotype who harbored several AML somatic mutations at similar frequency to the original AML patient newly presented in this article (Figures 1A–1C). These AML iPSCs similarly demonstrated a myeloid-restricted differentiation phenotype similar to that of the AML patient (Figure 1D). Nevertheless, further work is needed to comprehensively determine which blood cancers and their subtypes are more susceptible to iPSC reprogramming and the biologic rationale for which mutations portend a refractory reprogramming outcome.

Second, technical variations in reprogramming methodologies are likely to lead to differences in the ability to reprogram a wider range of blood cancers. One of the major technical barriers is the ability to titrate cell proliferation rates to ensure an optimal reprogramming outcome. Cell proliferation and active cell division are critical for successful integration of reprogramming vectors and subsequent iPSC generation. For several blood cancer types, the ability to proliferate primary patient cells in vitro is a significant challenge. This observation is particularly the case for AML patient samples with limited ex vivo proliferation potential. Multiple prior attempts to proliferate and expand primary AML cells in vitro have been largely unsuccessful. Recently, AML iPSCs were successfully generated using a novel stromal cell–conditioned media system

A

Sample	Description	Source	Mutation	Cytogenetics	iPSCs	AML iPSCs	Reprogramming Frequency (%)
SU444	60M de novo AML (FAB=M4)	PB (bulk) 29% blasts	DNMT3a R882H ERBB4-N390D FLT3-ITD	46XY	6	Yes	0.00003

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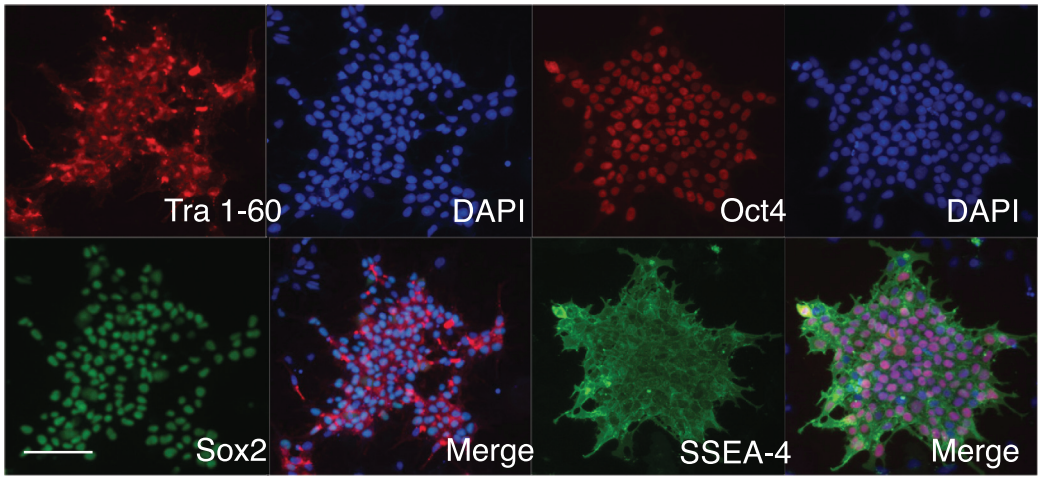

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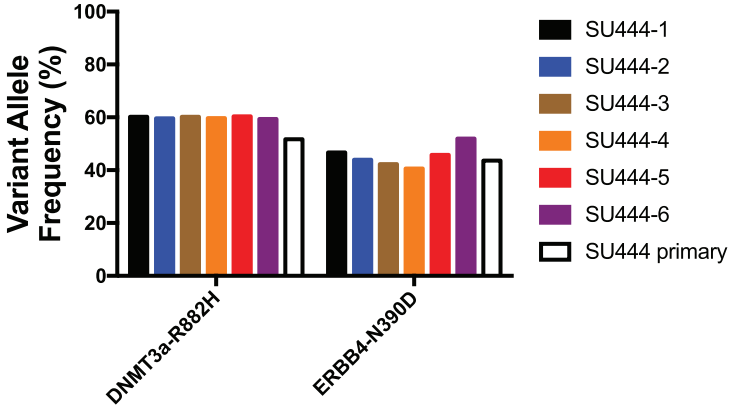

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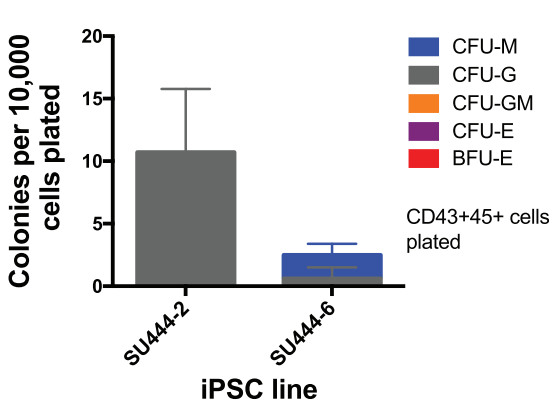

Figure 1. Successful reprogramming of a normal karyotype AML patient to iPSCs. **(A)** Patient characteristics and iPSC reprogramming frequency of a normal karyotype primary AML patient (SU444). Briefly, bulk AML SU444 patient peripheral cells were reprogrammed into iPSCs through Sendai virus containing Sox2, Klf4, Oct4, and c-Myc transcription factors as described in Chao et al. [3]. FAB=French–American–British classification. The iPSC column refers to the number of iPSCs generated from reprogramming. AML patient samples were obtained according to institutional review board (IRB)-approved protocols (Stanford University IRB nos. 18329 and 6453 after informed consent and reprogramming of AML samples conducted under Stanford IRB no. 28197). **(B)** Pluripotent markers on AML-derived iPSCs shown by immunofluorescence according to protocol described in Chao et al. [3]. Scale bar is 200 μM (white). **(C)** Targeted DNA sequencing of the primary AML patient-derived (SU444 primary) and AML-derived iPSCs are shown. Targeted amplicon sequencing of leukemic mutations was performed as described in Corces-Zimmerman and Majeti [23]. **(D)** AML-derived iPSCs were differentiated into $CD34^{+}CD45^{+}$ hematopoietic cells plated in methylcellulose to evaluate hematopoietic colony formation as described in Chao et al. [3]. Two iPSC lines are shown, demonstrating a myeloid-restricted differentiation profile and recapitulating the myeloid-restricted differentiation of the primary AML sample.

that enabled the proliferation of primary AML cells in vitro [3]. In this study, a doubling time of 24–48 hours was found to be an ideal rate of proliferation for iPSC reprogramming. The proliferation status of primary AML cells was a key factor in the ability to generate iPSCs and may help to explain the success with MLL-rearranged AML samples [3], which seem to be more able to proliferate in vitro. A second barrier may be due to the specific reprogramming vector strategy used to generate iPSCs. Although the use of the reprogramming factors (Oct4, Sox2, Klf4, and c-Myc) has generally been consistent for cancer-derived iPSC generation, the use of the vectors to deliver these factors has varied. These vectors include integrating viruses (retroviral, lentiviral) as well as nonintegrating (Sendai-virus based, episomal) approaches. Although patient-derived iPSCs have been generated from all of these vector types (Table 1), the use of a specific vector type may enhance the probability of reprogramming success in certain blood cancers.

Third, whereas patient-derived iPSCs carry the promise of a personalized medicine approach for patient-specific disease modeling and therapeutic targeting, this application is likely more challenging with cancer-derived iPSCs. This challenge is due to three key barriers. First, the amount of time and associated costs needed to derive individual iPSCs from cancer patients is likely too long and costly for real-time patient decision making. Generation of iPSCs from patient samples typically takes few to several months, making real-time diagnostic and therapeutic decision making not feasible. Nevertheless, biologic and therapeutic insights from iPSC lines can be validated in primary patient samples, which can then be applied to patients of a similar genetic makeup, as described in prior studies [3,10]. Second, the variability of successful iPSC generation by cancer type makes it challenging to apply this approach to a broad group of patients. Third, the low frequency of cellular reprogramming can lead to a lack of full characterization of a patient's genetic mutational landscape. Indeed, reprogramming frequencies can range from 0.00001% to 0.001% [3,18] (Figure 1A). Improvements in reprogramming technologies and expansion of primary cancer patient samples should help to overcome some of these barriers.

In summary, whereas iPSCs have been successfully generated in multiple blood cancers, barriers remain for their application across a wider range of hematologic malignancies. Ongoing efforts are needed to elucidate avenues to overcome barriers to cellular reprogramming in certain cancers.

Summary

The initial discovery in 2007 by Yamanaka and colleagues of inducing differentiated cells to pluripotency has led to a robust exploration of iPSCs as unique models of human disease. In the last decade, this effort has crossed over into modeling human cancer. A robust set of studies has demonstrated that iPSCs can be successfully generated in multiple hematologic malignancies with the ability to model key aspects of malignant hematopoiesis, both in vitro and in vivo. These models have uncovered unique biologic and therapeutic insights, including the impact of epigenetic reprogramming on cancer initiation, modeling the dynamics of genetic subclones of disease, and discovery of novel therapeutics through iPSC-based drug-screening platforms. Although iPSC-based models of cancer have demonstrated proof of concept as a novel and effective model of disease, several questions remain in this field that may limit its widespread utility, including whether all cancer types can be successfully reprogrammed to iPSCs. Nevertheless, the insights generated by these studies emphasize the importance of incorporating iPSC-based models into the evolving arsenal of research tools available to further elucidate our understanding and therapeutic targeting of cancer.

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Conflict of interest disclosure

MPC is an employee and equity holder of Forty Seven, Inc. RM is a consultant, equity holder, and member of the board of directors for Forty Seven, Inc.

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