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Modeling malignancies using induced pluripotent stem cells: from chronic myeloid leukemia to hereditary cancers

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Over the last decade, the possibility of reprogramming malignant cells to a pluripotent state has been achieved in several hematological malignancies, including myeloproliferative neoplasms, myelodysplastic syndromes, and chronic myeloid leukemia (CML). It has been shown that it is readily possible to generate induced pluripotent stem cells (iPSCs) from several types of primary CML cells and to generate progenitors and differentiated cells with variable efficiency. Although these experiments have brought some new insights in the understanding of CML pathophysiology, the ultimate goal of generating induced leukemic stem cells (LSCs) with long-term multilineage potential has not yet been demonstrated. Experiments under way will determine whether additional signaling events are required to induce the emergence of bona fide LSCs. However, iPSC modeling offers the unique possibility to generate pluripotent cells harboring cancer-predisposing mutations using patient-derived noncancerous cells, as has been shown in Li–Fraumeni syndrome, BRCA-1 associated breast carcinomas, or RET-mutated medullary thyroid carcinomas. In these conditions, mutated iPSCs can then be used to study the mutational history that precedes the appearance of the malignant transformation and to develop novel drug-screening strategies. The ability to induce a successful differentiation program toward the tissue in which a given cancer develops or to generate tissue-specific cancer organoids in which the full oncogenic potential can be revealed remains a major challenge in the field. Similarly, in hematological malignancies, a significant hurdle remains due to the lack of adequate technology to induce the emergence of leukemic cells that resemble LSCs, which hinders our ability to study the mechanisms of therapy resistance. © 2019 Published by Elsevier Inc. on behalf of ISEH – Society for Hematology and Stem Cells.

The seminal discovery of cell reprogramming in 2006 by Shinya Yamanaka opened up novel and unprecedented perspectives, not only in the field of biology in general, but also in all fields of medicine, with almost endless possibilities in terms of therapeutic applications [1]. Although the use of induced pluripotent stem cells (iPSCs) appeared initially as a technique that would be widely applicable alternatives to embryonic stem cells (ESCs), several hurdles remain to be solved for the use of iPSCs and their derivatives in regenerative medicine. One of the major problems in this field has been the lack of robust differentiation protocols toward a given tissue with generation of functional cells. Indeed, in the field of hematopoiesis and in other fields, the immature status of several types of differentiated cells such as erythrocytes represents a significant "bottleneck' for disease-modeling strategies. The second concern is the genetic stability of the iPSC lines during several passages with the possibility of occurrence of somatic mutations. The immunogenicity of the cells is also a concern for regenerative medicine approaches. Conversely, it became rapidly apparent that iPSC technology represents a highly spectacular tool of disease modeling in all fields of medicine from neurology to cardiovascular disorders [2]. In the field of cancer, the first hematopoietic malignancy to be studied was chronic myeloid leukemia (CML) and this field progressed rapidly with development of exciting novel models of myeloproliferative neoplasms [3,4], myelodysplastic syndromes (MDS) [5], acute myeloid leukemia (AML) [6], and more recently in solid tumors and specifically in hereditary cancers. In MDS, the

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reprogramming technology allowed the generation of hematopoietic cells from low- and high-risk patients and studies have shown the possibility of testing the effects of the differentiation agent 5-azacytidine [7]. We discuss in this review recent developments in the field of CML and hereditary cancers.

Malignancies and the pluripotent state: common grounds

Several lines of evidence suggest that cancer development and cellular reprogramming share many features and signaling pathways. Indeed, since the initial clonal theory of cancer [8], the concepts of cancer development and evolution have evolved considerably. The heterogeneity of cancer was initially thought to be the result of successive mutational events leading to a growth advantage gained by the most aggressive subclones capable of metastatic dissemination. In the tumor microenvironment, all cells were thought to be equal in terms of tumorigenic potential. With the development of stem cell assays and markers, initially in hematopoietic malignancies and later in several types of solid tumors, it has been established that tumors contain functionally distinct populations, one population able to self-renew and another undergoing differentiation and generating most of the bulk of the tumor. The "cancer stem cell hypothesis" was initially confirmed in hematopoietic disorders including myelodysplasia and chronic myeloid leukemia by the demonstration of the clonal nature of these disorders [9,10]. The cancer stem cell concept in solid tumors required the development of novel markers and assays, which led to their discoveries in glioblastoma [11,12], colon carcinomas [13], and breast carcinomas [14]. In particular, it has been shown that a small population of cells isolated in human tumors using CD133/ALDH expression or biochemical characteristics (Hoechst efflux) was enriched in cells with self-renewal potential, proliferation potential, and resistance to chemotherapies. The mechanisms of generation of cancer stem cells in a solid tumor has been a subject of a debate because this could be the result of the transforming event taking place in a normal, self-renewing stem cell or, alternatively, a transforming oncogenic event may induce a de-differentiation potential in a normal differentiated cell, which acquires stem cell potential using transcription machinery involved in epithelial–mesenchymal transition [15]. It has also been shown that the most aggressive cancer cells express embryonic genes [16–18]. Similarly, transcriptome analyses performed in this setting have shown that glioblastoma progenitor cells expressed levels of Oc4 and Sox2 similar to those found in human ESCs [19].

The epigenetic resetting that occur during the tumor development is a feature presenting high similarity with the adult cell reprogramming [20]. Conversely, all "Yamanaka" factors (C-Myc, Klf4, Sox2, and Oct4) have established oncogenic potential and are expressed in several types of cancer cells. Lin28, another reprogramming factor, is expressed also in cancer cells and in blast crisis of CML [21]. The use of iPSCs could therefore be of major interest to study in vitro the signaling pathways involved in cancer [22]. However, it appeared that reprogramming cancer cells are not straightforward due to signaling barriers such as TP53, the deletion of which increases the efficiency of reprogramming [23]. If the reprogramming approach is successful, then the second important requirement is the ability to induce a differentiation of the tumor-derived iPSCs toward the tissue in which the primary cancer occurs in order to recapitulate the behavior observed in primary cancer cells. A hematopoietic malignancy such as CML was an excellent candidate disorder for cell programming because techniques of hematopoietic differentiation from both murine and human ESCs and iPSCs have been established. However, the generation of definitive hematopoiesis with long-term repopulating activity remains a challenge from both ESCs and iPSCs.

Modeling CML using iPSCs

CML is a hematopoietic stem cell malignancy arising from a primitive hematopoietic stem cell [24]. The major interest in the CML stem cells in recent years is related to the fact that, despite the major success obtained by the tyrosine kinase inhibitor (TKI) therapies, the most primitive leukemic stem cells are resistant to them [24], leading to relapses upon TKI cessation [25]. Similarly, resistances to TKI therapies occur, leading to progression of the disease on therapy. CML stem cells are difficult to isolate and expand and, despite some recent cell surface markers such as CD26 [26], there have been no specific biomarkers that have been universally validated in CML [24]. In addition, available in vitro or in vivo mice models do not recapitulate the evolution of human CML, especially with regard to the first stages of the disease and its natural history. To elucidate these questions, we and others turned to model systems to generate ESC-based [27,28] or, more recently, iPSC-based experimental models. One of the expectations with the use of CML iPSC models was the fact that, as demonstrated in murine ESC system, the expression of oncogenic BCR-ABL would lead to the generation of a long-term repopulating ability and therefore a leukemic stem cell (LSC) potential that has not been shown so far with the use of either human ESCs or iPSCs.

The demonstration of the feasibility of programming CML cells was rapidly shown using the blast crisis cell line K562 from which BCR-ABL-expressing $CD34^+$ cells, albeit with a very low yield, have been obtained [29]. Further work has confirmed the possibility of cell reprogramming either from total bone marrow, peripheral blood mononuclear cells (PBMCs), or from Philadelphia chromosome 1-positive ($Ph1^+$) $CD34^+$ cells

from patients with CML [30–33]. The analysis of these cell lines has shown the feasibility of generating from the $Ph1^+$ iPSCs, clonogenic progenitors, and differentiated hematopoietic cells expressing BCR-ABL. The iPSC lines expressing BCR-ABL were found to be resistant to imatinib, as might be expected from their pluripotent state, but they acquired partial imatinib sensitivity when induced to differentiation [32,33]. The analysis of iPSC-derived CML cells has shown the validity of the novel signaling pathways discovered by this strategy, such as olfactomedine [34] or ADAM8 [35]. One major question of major interest to address with the use of CML iPSCs was the possibility of generating LSCs, which are known to be highly resistant to TKIs [36]. The possibility of obtaining these cells, which are difficult to isolate from primary CML marrow, would be of major interest for LSC-based drug-targeting purposes. In our center, we have reprogrammed leukemic cells from several patients with CML at diagnosis before adm [37]. To analyze the in vitro potential of CML iPSCs, we have used $CD34^+$ cells from a patient whose leukemic cells harbored both a Ph1 chromosome and a V617F JAK2 mutation [33]. After successful reprogramming and characterization, the "double-positive iPSCs" were used to determine their hematopoietic potential using colony-forming cell and long-term culture-initiating cell (LTC-IC) assays combined with their drug sensitivity. Interestingly, we have shown that, compared with human ESCs, BCR-ABL/Jak2-mutated iPSCs exhibit an increased LTC-IC potential [33]. It is difficult to determine whether this potential was due to the presence of both oncogenes or to BCR-ABL alone, but this tool allowed us to validate the use of hematopoietic cells derived from iPSCs for drug-screening purposes. We have indeed shown in these BCR-ABL-expressing JAK2-V617F-mutated progenitors that pimozide, a STAT5 phosphorylation inhibitor, exhibited synergistic inhibitory activity with imatinib [33]. The possibility of generating from CML iPSCs, or "LSC-like" cells, was also shown by another study [34], which also showed that these cells were resistant to imatinib [34].

Therefore, the generation of "LSC-like" cells from CML iPSCs with either phenotypic markers or LTC-IC generating potential could be shown, but so far there has been no data showing that CML-iPSCs could generate long-term repopulating LSCs in immunodeficient mice. In our studies using several iPSC lines obtained from several patients, we have transplanted into NSG mice, either by intravenous or intrafemoral injection, embryoid bodies, or $CD34^+$ cells derived from CML iPSCs, but we have not observed a long-term repopulating potential. It appears that the human CML iPSC misses a signaling link that is present in murine pluripotent cells transduced with BCR-ABL. This deceiving result compared with murine ESCs expressing BCR-ABL [27,28] could be due to the fact that, in the murine experimental systems, BCR-ABL expression is under the control of strong promoters as opposed to human CML iPSCs. The lack of an adequate "niche" allowing the emergence of a primitive LSC potential could also explain these results.

Indeed, it has also been shown that teratomas generated by injection of iPSCs in NSG mice can be a permissive microenvironment for spontaneous in vivo generation of hematopoietic cells, which could migrate to the bone marrow with the potential to give rise to serial transplantations [38]. It remains to be determined whether CML iPSCs can acquire LSC potential in the same conditions. Future transcriptome experiments in CML iPSCs will also aim to identify a signaling pathway that could enhance this potential. The aryl hydrocarbon receptor pathway that we identified as a major regulator of malignant progenitor proliferation in CML could be also a druggable target in CML iPSCs to enhance LSC activity [39].

The second important question to validate the use of CML iPSCs in disease modeling was the genetic stability of the cell lines compared with parental primary leukemic cells. Although previous studies have shown that most of the genetic abnormalities observed in iPSC lines were present in the original target cells and disappeared during increased passages [40], this question was not addressed in the setting of a neoplastic iPSCs. We studied this question by performing whole-genome sequencing analysis of our iPSC lines harboring both Ph1 chromosome and V617F JAK2 mutations compared with cryopreserved primary leukemic cells from the same patient. This analysis showed a highly preserved genomic structure during and after reprogramming, with very few additional mutations, suggesting excellent "genocopy" of the primary leukemic cells during iPSC generation [33]. The demonstration of the adequate "capture" of genomic alterations present in primary leukemic cells upon cellular reprogramming was an important step forward in validating the possibility of using the iPSC-derived CML models with regard to the CML pathophysiology and for future studies. Figure 1 shows some achievements and summarizes future challenges in the field of CML using iPSC technology.

Modeling hereditary cancers using iPSCs: from predisposition to "disease in a dish"

As opposed to hematopoietic malignancies, in particular CML and other myeloproliferative neoplasms, reprogramming cancer cells to pluripotency has proven to be difficult. Early experiments of nuclear transfer performed before cell reprogramming technologies have shown that it was not possible to reprogram nuclei of several tumors (breast, lymphoma) to pluripotency except for melanoma,

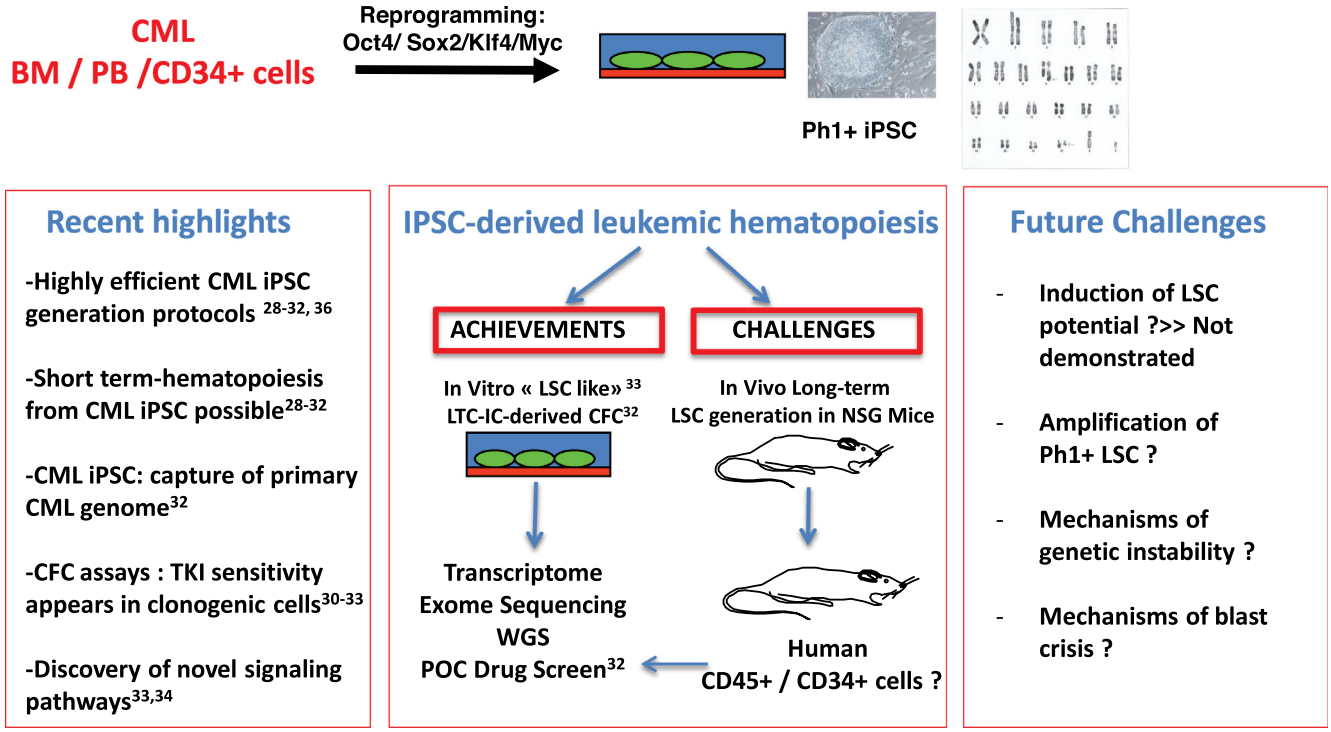

Figure 1. Schematic representation of achievements in disease modeling for CML using patient-derived IPSCs. Several important findings have been reported with regard to CML pathophysiology, but the major "bottleneck" remains the inability to generate long-term LSCs. *POC*=proof of concept.

from which a PSC line could be developed by nuclear transfer [41]. These early results suggested that, at least in some cancer cells, the presence of an established malignant phenotype was compatible with genome reprogramming. The rarity of this event could be due to the presence of complex abnormalities present in these cells. The use of iPSC technology later confirmed the relative inefficiency of cell programming in malignant cells from solid tumors in general. In the case of pancreas cancer, an iPSC line from a pancreas adenocarcinoma cell could be obtained with a very low efficiency using a doxycycline (DOX)-inducible gene transfer system [42]. However, this iPSC cell line required the continuous expression of pluripotency factor expression (because it required the addition of DOX in the cell culture) and probably does not reflect a true "pancreas cancer iPSC" [42]. The major inefficiency of reprogramming of a bona fide cancer cell to pluripotency could also be related to the fact that cancer cells could require additional reprogramming factors compared with normal cells, as has been shown in glioblastoma [43]. The ability to reprogram solid tumors for identification of early stages of oncogenesis remains a challenge that will require further efforts under way in several laboratories. The difficulty of obtaining "cancer iPSCs" from transformed cells stimulated efforts to generate in vitro cancer models by using gene editing technology from normal iPSCs [44]. Figure 2B summarizes different strategies that can be used to generate a "cancer iPSC" in vitro, but the possibility of recreating a tumor phenotype reminiscent of human cancer remains to be confirmed in future studies.

As opposed to sporadic cancers, which accumulate genomic abnormalities leading to their discovery, hereditary cancers are characterized by long-lasting latency in the presence of a germline genomic event known to predispose to the occurrence of cancer. In this context, cell programming represents a fascinating modeling tool because a germline oncogenic mutation or deletion of a tumor suppressor gene is already present in an otherwise healthy donor tissues. This will allow the determination of the cell of origin of initial neoplastic transformation and the tissue specificity of a given cancer by the theoretically unlimited differentiation potential of pluripotent cells. Mutations or deletion in genes such as BRCA1 and TP53 represent one of the earliest events in familial breast cancers and Li–Fraumeni syndrome, respectively. Although, in these disorders, the classical "second hit" concept refers to the loss of a second allele, the signaling events induced in the oncogenic transformation are probably much more complex, implicating epigenetic abnormalities. Similarly, the tissue specificity of the cancer predisposition remains poorly elucidated. One of the prerequisites of the model is the ability to induce from iPSCs a tissue or organoid close to the organ in which the cancer

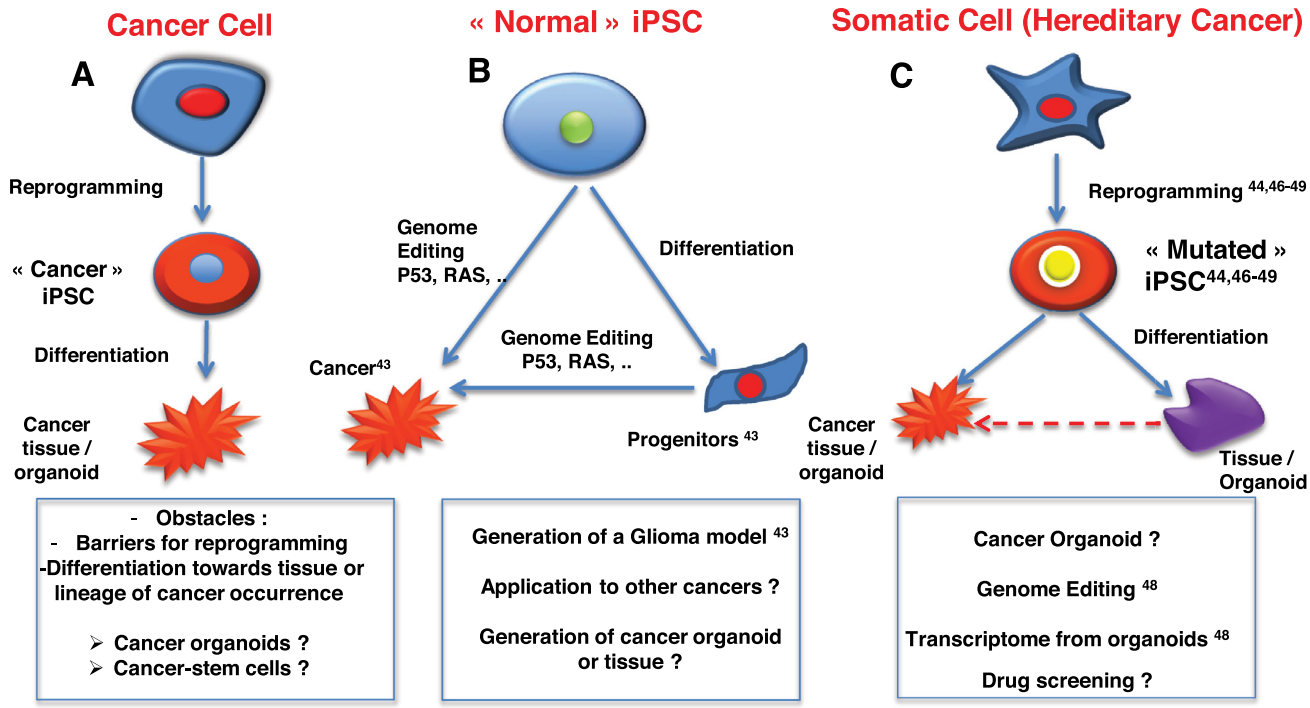

Figure 2. Schematic representation of different strategies used in cancer modeling using IPSC technology. **(A)** Reprogramming of bona fide cancer cells to a pluripotent phenotype has proven to be difficult, probably due to genetic and epigenetic modifications accumulated in cancer cells. **(B)** These difficulties could be experimentally circumvented using genome editing of normal iPSCs to induce a malignant phenotype, such as what was reported in a model of experimental glioma [43]. **(C)** In the context of cancer predisposition syndromes, cell reprogramming of a healthy tissue carrying an oncogenic mutation has been possible in several types of hereditary cancers [44,46–49]. These cells can then be used to perform genome editing to determine the effects of gene correction [48] or to generate organoids in which the genomic events required for full transformation can be tested.

arises in vivo. The possibility of modeling Li–Fraumeni syndrome using iPSCs has been demonstrated using patient-specific iPSCs with an osteosarcoma phenotype generated after osteoblastic differentiation [45]. It has been shown that Li–Fraumeni-syndrome iPSC-derived osteoblasts present features of Li–Fraumeni syndrome osteosarcoma both phenotypically and using gene expression analyses [45]. In iPSC-derived osteosarcoma cells, there was a major downregulation of the H19 gene and its overexpression reverted the tumorigenic potential of osteosarcoma cells [45]. These experiments were clearly the first demonstrating cell programming in the setting of a hereditary cancer.

BRCA1-mutated breast cancer represents another attractive target for cell-programming strategies. BRCA1, the "guardian of genome integrity," plays a critical role in DNA repair and its alteration by mutations or deletion is associated with the predisposition of the occurrence of familial breast and ovarian cancers. Similar to Li–Fraumeni syndrome, the possibility of obtaining BRCA1-altered iPSCs offers the possibility of studying secondary genetic events associated with BRCA1 abnormalities. For example, it has been shown that the functional abrogation of the second allele of BRCA1 could be, albeit infrequently, achieved by an epigenetic mechanism [46]. The availability of the BRCA1 iPSCs could allow to test in vitro and in vivo the phenotypic abnormalities observed by modification of gene expression or genome editing. The possibility of generating BRCA1=iPSCs has been shown by Soyombo and colleagues [47] and our group [48]. This unique BRCA1-deleted iPSC from a patient with exon 17 deletion exhibited all features of pluripotency using in vitro and in vivo assays with no evidence of malignant tumor in teratomas generated in NSG mice [48].

One other interesting target for cell programming in the case of cancer predisposition is the multiple endocrine neoplasia 2A (MEN2A) syndrome due to germline mutation of c-RET and including essentially medullary thyroid cancer and pheochromocytoma [49]. c-RET encodes for a transmembrane tyrosine kinase and its activation occurs through heterodimerization with the glial derived neurotrophic factor (GDNF) receptor in the presence of the ligands of the GDNF family. The oncogenic mutant form has increased tyrosine kinase activity, leading to cell proliferation in the absence of the ligand. The disease can vary in severity as a function of the mutation, the most aggressive disease being due to the c-RET mutant M918T.

We had the opportunity to generate iPSCs using PBMC of three patients with various c-RET mutations followed by genomic and functional analyses [50,51]. All three iPSC lines with RET mutations expressed pluripotency markers and led to teratoma formation with trilineage differentiation when injected into immunodeficient NSG mice. Although we did not observe any neoplastic phenotype either in vitro or in vivo, we were able to derive isogeneic corrected iPSCs (RET-Y634C) in the case of a "high-risk" C634Y-mutated iPSC using CRISPR/CAS9 technology [50]. Both mutated and corrected iPSC lines were subjected to exome sequencing to evaluate potential off-target events, which were minimal [50]. Interestingly, integrative differential gene expression analysis of RET-C634Y, RET-Y634C, and RET-wild-type iPSCs revealed activation of the early growth response 1 (EGR1) transcriptional program specifically in RET-C634Y-mutated iPSCs, which had been previously suggested based on studies using cancer cell lines.

The bioinformatics analysis revealed that EGR1 elicited a transcriptional program specifically in iPSCs harboring oncogenic RET in two different RET-mutated iPSC lines [50]. Although we could not generate terminally differentiated calcitonin-secreting C cells, these findings strongly confirmed the feasibility of target discovery strategies using RET-mutated iPSCs and showed as proof of concept that iPSCs can be used as a potent platform in hereditary cancers. One important additional advantage using iPSCs in the context of hereditary neoplasms is the ability to develop in vitro 3D organoids, for instance, using iPSCs derived from patients with BRCA1-mutated breast or c-MET-mutated renal carcinomas. The possibility of modeling these cancers using iPSC technology offers novel exciting perspectives because the initial genomic abnormality is carried not only at the pluripotent stage, but also during tissue specification and cellular differentiation. Recent data showed the feasibility of the modeling familial adenomatous polyposis characterized by germline mutation of the WNT signaling pathway using iPSC-derived colonic organoids, which could then be used as a drug-screening platform [52].

Perspectives

The possibility of modifying cell fate by reprogramming forever changed the field of cell biology with potential major therapeutic perspectives in all fields of medicine. The recent development of iPSC-derived organoid technology combined with CRISPR-CAS9 revolution allows unprecedented innovations in the field of disease modeling with the generation of cancer models from both hematological and nonhematological malignancies. In the field of hematological malignancies, one important challenge remains the possibility of generating a "true" LSC with long-term repopulating potential both in CML and other myeloproliferative and myelodysplastic disorders. In the field of solid tumors, iPSC technology can be of major interest in modeling hereditary cancers and progress will be closely linked to that of organoid technology. Reprogramming a cancer cell with a fully transformed phenotype is still a challenge, but will certainly be possible with an improved understanding of the epigenetics of the cancer cell and will likely open up novel perspectives for future cancer therapy approaches.

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