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Opinion

Modeling Cancer with Pluripotent Stem Cells

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The elucidation of cancer pathogenesis has been hindered by limited access to patient samples, tumor heterogeneity, and the lack of reliable model organisms. Characterized by their ability to self-renew indefinitely and differentiate into all adult cell lineages of an organism, pluripotent stem cells (PSCs), including ESCs and induced pluripotent stem cells (iPSCs), provide a powerful and unlimited source to generate differentiated cells that can be used to study disease biology, facilitate drug discovery and development, and provide key insights for developing personalized therapies. This article reviews the recent developments and technologies converting PSCs into clinically relevant model systems for cancer research.

Modeling Disease with Pluripotent Stem Cells

In 1998, Thomson and colleagues isolated human **ESCs** (see Glossary) from blastocysts and developed a defined culture system to maintain the cells *in vitro* [1], opening a new avenue for medical research. Later, in 2006–2007, a breakthrough by the laboratories of Yamanaka and Thomson heralded the development of a new kind of pluripotent cells – **induced pluripotent stem cells (iPSCs)** [2–4]. Both groups demonstrated that somatic cells (e.g., dermal fibroblasts and peripheral blood) could be **reprogrammed** to an ES-like cell state using a defined transcriptional factor cocktail (Yamanaka's OCT4, SOX2, KLF4, c-MYC; or Thomson's OCT4, SOX2, NANOG, LIN28) [5]. Over the past decade, subsequent advances facilitated the generation of iPSCs with chemicals, microRNA and modified RNA, or other gene delivery systems (retroviruses, adenoviruses, Sendai virus, transposons, and plasmids) [5]. Applications for iPSCs include regenerative medicine, disease modeling, drug screening, and personalized therapy.

The unique combination of pluripotency and self-renewal distinguishes **PSCs**, including both ESCs and iPSCs, from all other cells (Figure 1A). The unlimited proliferative potential of these undifferentiated cells provides an arbitrarily large source of experimental material, while their pluripotency allows them to be coaxed into forming all adult tissue types. Well-defined protocols, including directed differentiation and **organoid** cultures have been developed to derive many major target tissues and cell types from PSCs of endodermal (liver, small intestine, stomach, thyroid, and lung), mesodermal (muscle, bone, cartilage, kidney, and blood), or ectodermal (epidermis, retinal, and cerebral tissue) lineages [6–8].

PSCs provide unparalleled advantages as a model system, allowing investigators to study a cell continuously from the moment it differentiates from a multipotent progenitor into a differentiated cell type of interest. The relevant genetic background for the model system can be introduced into PSCs using two primary strategies. In one approach, somatic cells from patients with genetic disorders are used to derive iPSC lines. These patient-derived iPSCs and their derivative differentiated tissues are then used to recapitulate a disease phenotype *in vitro* or shed light on

Trends

Modeling cancer using pluripotent stem cells (PSCs) overcomes several disadvantages of current model systems, including limited accessibility of patient samples, tumor heterogeneity, and differences between species.

PSCs provide a powerful and unlimited source to generate differentiated cells that can be used to elucidate disease pathogenesis, drug discovery and development, and advance personalized health care.

Both patient-derived induced PSCs and engineered ESCs completely phenocopy cancer features, suggesting that PSCs can serve as a useful *in vitro* human cancer model.

Currently evolving methodologies for gene expression manipulation, genome editing, and cell differentiation facilitate the application of PSCs to cancer research.

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disease-relevant mechanisms [9]. This approach has been applied successfully to study the genetic causes of neurodegeneration [10–12], mental disorder [13], heart disease [14–17], and metabolic disorders [18].

Alternatively, a genetic disease trait can be directly introduced into PSCs. This approach is aided greatly by recent major developments in gene delivery systems such as helper-dependent adenoviral vectors [19], adeno-associated viruses [20], gene manipulation approaches (RNAi [21,22] and piggyBac transposases [23]), and genome-editing tools, such as **zinc-finger nuclease** [23–25], **transcription activator-like effector nucleases (TALENs)** [26,27], and **clustered, regularly interspaced, short palindromic repeat/Cas9 (CRISPR/Cas9)** [28,29]. These technologies allow introducing alterations (deletions, amplifications, mutations, or gene fusions) into ESCs or iPSCs of an arbitrary genetic background, allowing studying human monogenic and complex diseases as the pathology develops (Box 1).

While the field of PSC-derived cancer research remains in its infancy, a number of PSC-derived cell lines have been generated to model disorders with a cancer predisposition (Table 1). Several groups have applied patient-derived iPSCs and/or engineered PSCs to phenocopy cancer features, explore disease mechanisms, and screen potential therapeutic drugs [30–34]. Their experience highlights the potential of human PSCs in cancer studies by overcoming limitations related to availability of patient samples or translation of results from animal models or cell lines with inappropriate genetic backgrounds. Here, we outline the existing PSC cancer models and their potential applications to understanding cancer biology. We discuss how recent developments (e.g., genome-editing and cell differentiation technologies) in PSCs have transformed our understanding of cancer biology and paved the way for new therapeutic strategies. Finally, we review some of the most promising model systems in which we anticipate this powerful technology will be applied.

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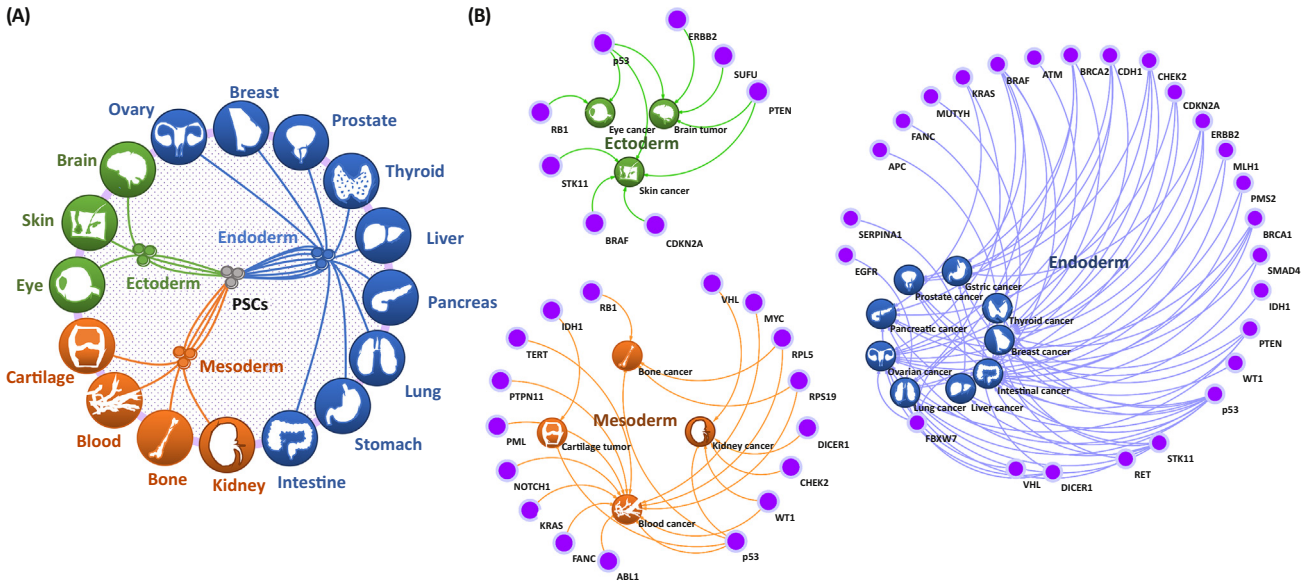

Figure 1. Application of Pluripotent Stem Cells (PSCs) to Study Cancer-Associated Genetic Alterations. (A) PSCs are characterized by their capability to differentiate into all derivative cell types of the three germ layers. PSCs can form blood, kidney, bone, and cartilage cells via the mesoderm; ovary, breast, prostate, thyroid, liver, pancreas, lung, stomach, and intestine cells via the endoderm; and brain, eye, and skin cells via the ectoderm. (B) Loss of tumor suppressor genes, such as p53 mutation; or acquisition of oncogenes, such as ERBB2 amplification or ABL1 translocation, results in both hereditary and sporadic cancers in ectodermal, mesodermal, and endodermal tissues.

Box 1. Tailoring the Pluripotent Stem Cell (PSC) Genome to Model Disease. Advanced genome-editing methodologies have now made it practical to tailor the human genome at the single-nucleotide level. Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered, regularly interspaced, short palindromic repeat/Cas9 (CRISPR/Cas9) have revolutionized the genome-editing field by allowing site-directed mutagenesis. A combination of a genomic localization domain with a domain conferring nuclease activity permits the introduction of a directed double-stranded break (DSB) at any site of interest. Site-directed mutagenesis without DSBs has recently become feasible for certain (cytosine to thymidine) 'base editing' using a CRISPR/Cas9 fused to cytidine deaminase [92]. By applying any of these approaches, it is now feasible to generate PSCs harboring specific mutations from a PSC line of choice or to correct any genomic alteration in patient-derived induced PSCs (iPSCs).

Correcting Mutations in iPSCs

Genome editing has been effectively applied to correct the genetic alterations in patient-derived iPSCs for both research and clinical applications. Correction of a mutation in a patient-derived iPSC can be used to generate an isogenic control and demonstrate a mechanistic link between a mutation and its downstream disease state. Importantly, it permits study from a genetic background in which disease penetrance has been established. It also may serve as a promising solution for transplantation-based therapies. For instance, ZFN–piggyBac-mediated correction of *SERPINA1* mutations in α1-antitrypsin-deficiency patients-derived iPSCs was shown to lead to the rescue of SERPINA1 structure and biological function [23]. TALEN-mediated gene-corrected X-linked severe combined immunodeficiency (SCID-X1) iPSCs demonstrated a rescue of defective hematopoietic differentiation [93]. CRISPR/Cas9–piggyBac-mediated correction of human hemoglobin beta (*HBB*) mutation in β-thalassemia patient-derived iPSCs restored the expression of HBB expression [94]. Similarly, positive–negative drug selection strategies combined with gene targeting on chromosome 21 have been demonstrated to correct trisomy in Down syndrome iPSCs [81]. While not yet ready for clinical practice, transplantation of differentiated tissue from these engineered iPSC lines back into their patient sources may permit a cure of the underlying disease, particularly in single-gene disorders that can be rescued by only a small amount of functional enzyme.

Inducing Mutations in Wild-Type PSCs

While iPSC models offer unique advantages for modeling a particular patient's genetic background, the diversity of their genetic origins complicates the integration and comparison of findings across multiple iPSC lines. By contrast, genome-edited PSCs based on extensively characterized lines are likely to be less variable and provide a more useful resource to understand central disease pathogenesis at the genomic scale. For instance, generation of a polycystic kidney disease (PKD)-linked *PKD1* or *PKD2* knockout in human ESCs results in cyst formation in kidney tubules in an organoid model, recapitulating the human phenotype [70]. Introduction of a long-QT syndrome (LQTS)-associated KCNH2 mutation in human ESCs leads to reduced current conducted by the HERG channel and prolonged action potential duration, recapitulating the LQTS phenotype [95].

In summary, early studies applying genomic editing to PSCs highlight the potential of this system for investigating disease pathogenesis and developing clinical cell therapies.

Modeling Cancer with Pluripotent Stem Cells

Over the past 40 years, researchers have used cancer cell lines, patient samples, and small organism models (e.g., fruit fly, zebrafish, and mouse) to study the molecular mechanisms of cancer initiation, progression, and metastasis, but the complexity of the cancer genome and differences among species frequently limit clinical translation. Although there are iPSC models for a number of genetic diseases that predispose to cancer, to date, relatively few of these systems have been used to explore mechanisms of oncogenesis. We discuss several examples of these pioneer models in the following sections.

Li–Fraumeni Syndrome

Li–Fraumeni syndrome (LFS) is an autosomal dominant inherited cancer syndrome that is characterized by early onset of a variety of tumor types, including soft-tissue sarcoma and osteosarcoma, breast cancer, brain tumors, leukemia, and adrenocortical carcinoma [35]. Our group established a model of LFS using patient-derived iPSCs to delineate mechanisms of mutant p53 in osteosarcoma [30]. In this system, osteoblasts differentiated from LFS iPSC-derived mesenchymal stem cells (MSCs) recapitulate osteosarcoma features, including

Glossary

Clustered, regularly interspaced, short palindromic repeat/Cas9 (CRISPR/Cas9): a genome-editing methodology based on the bacterial acquired immune system. Functioning in bacteria as a means of resistance to exogenous genetic elements similar to RNA interference in eukaryotic cells, it recognizes and cleaves DNAs based on a target RNA sequence. CRISPR systems depend on CRISPR RNA (crRNA) and *trans*-activating crRNA (tracrRNA) for sequence-specific silencing. There are three types of CRISPR/Cas9 systems. The type II system used in Cas9 and its variants serves as an RNA-guided DNA nuclease or nickase that cleaves DNA upon crRNA–tracrRNA target recognition. The ease with which constructs targeting desired genomic loci can be generated has made this technology the tool of choice for many seeking to perform genome editing.

ESCs: pluripotent stem cells are derived from the inner cell mass of the blastocyst, an embryo at the preimplantation stage. These cells are capable of proliferating and dividing without differentiating for a prolonged period in an *in vitro* tissue culture environment.

Induced pluripotent stem cells: pluripotent stem cells derived from differentiated somatic cells through somatic reprogramming by defined factors (e.g., OCT4, SOX2, KLF4, and c-MYC).

Organoid: a collection of multiple organ-specific cells cultured in a 3D system that self-organize into organ-bud structures. 3D-cultured organoids mimic the microanatomy of organs and are capable of recapitulating specific organ functions, enabling experimental study of otherwise inaccessible tissue.

Pluripotent stem cells: cells with equivalent characteristics to the inner cell mass of the blastocyst-stage embryo. Pluripotent stem cells are capable of differentiating into any cell type and give rise to all adult tissues (pluripotency) and extensively replicate without differentiation and/or senescence (self-renewal).

Reprogram: the process of converting one specific cell type to another. It includes the conversion of somatic cells (e.g., dermal fibroblasts)

Table 1. Established PSCs Models of Cancer or Diseases That Predispose to Cancer [a]

Disease	Inheritance	Prevalence	Defected Gene	Cancer Type	Key Model Findings in Cancer	Refs
Alpha-1 antitrypsin deficiency	Autosomal co-dominant	1 in 1500 to 3500	*SERPINA1*	Liver cancer	N/A	[18,23,77]
Ataxia-telangiectasia	Autosomal recessive	1 in 40 000 to 100 000	*ATM*	Leukemia and lymphoma	N/A	[78]
Diamond–Blackfan anemia	Autosomal dominant	1 in 5 000 000 to 7 000 000	*RPL5*, *RPL11*, *RPL35A*, *RPS7*, *RPS10*, *RPS17*, *RPS19*, *RPS24*, and *RPS26*	Osteosarcoma	N/A	[79,80]
Diffuse intrinsic pontine gliomas	Sporadic	1 in 250 000 to 500 000	*H3.3*	Brain tumor	Undifferentiated epigenome structure and a primitive stem cell gene signature in NPCs with H3.3K27 M expression, PDGFRA activation, and p53 loss	[33]
Down syndrome	Sporadic	1 in 800	Chromosome 21	Leukemia	N/A	[11,81]
Dyskeratosis congenita	Autosomal dominant or recessive	1 in 1 000 000	*DKC1*, *TERC*, *TERT*, and *TINF2*	Leukemia	N/A	[82,83]
Dystrophic epidermolysis bullosa	Autosomal recessive	1 in 150 000 to 1 000 000	*COL7A1*	Skin cancer	N/A	[84,85]
Fanconi anemia	Autosomal recessive	1 in 160 000	*FANC* genes, *BRCA2*, *BRIP1*, *PALB2*, and *RAD51C*	Leukemia	N/A	[86]
Glioblastoma multiforme	Sporadic	1 in 33 000 to 50 000	*EGFR*, *PIK3CA*, *PTEN*, and *TP53*	Brain tumor	Elevated PAX7 and GBM-associated gene signature in PTEN-deficient NSCs	[34]
Li–Fraumeni syndrome	Autosomal dominant	1 in 20 000	*TP53* and *CHEK2*	Osteosarcoma, breast cancer, brain tumor, and soft-tissue sarcoma	Impaired expression of H19 and osteosarcoma signature in LFS osteoblasts	[30]
del(7q)-Myelodysplastic syndrome	Sporadic	1 in 100 000	Chromosome 7q	Leukemia	Impaired myeloid lineage differentiation in del(7q) iPSCs dependent on HIPK2, ATP6V0E2, LUC7L2, and EZH2	[32]

to pluripotent stem cells and the conversion of one type of somatic cell to another.

Transcription activator-like effector nucleases (TALENs): a genome-editing methodology based on fusions of the *Fok*I DNA nuclease and a TAL effector DNA-binding domain derived from *Xanthomonas* bacteria. Engineered TAL effectors are able to bind to any specific DNA sequence, permitting *Fok*I nuclease activity at a desired DNA location. TAL effectors contain highly conserved 33–34 amino acid repeat domains that recognize a specific base pair. TALENs induce target double-stranded breaks to facilitate homologous recombination and enable customized genome alterations. TALENs are believed to have greater precision for genome editing than zinc-finger nuclease and clustered, regularly interspaced, short palindromic repeat/Cas9.

Zinc-finger nuclease: a genome-editing methodology based on a fusion of the *Fok*I restriction enzyme with a Cys2His2 zinc-finger DNA-binding domain. Zinc-finger nucleases were among the first available tools to perform genome editing.

Table 1. (continued)

Disease	Inheritance	Prevalence	Defected Gene	Cancer Type	Key Model Findings in Cancer	Refs
Noonan syndrome	Autosomal dominant	1 in 1000 to 2500	*PTPN11, BRAF*, *KRAS*, *NRAS*, *RAF1*, and *SOS1*	Leukemia	Proliferation of $CD33^+$ myeloid cells and elevated miR-15a and miR-223 in NS/JMML hematopoietic cells	[31]
Polycythemia vera	Sporadic	1 in 2000	*JAK2* and *TET2*	Leukemia	N/A	[87]
Shwachman–Diamond syndrome	Autosomal recessive	1 in 100 000 to 1 000 000	*SBDS*	Leukemia	N/A	[11,88]
Werner syndrome	Autosomal recessive	1 in 200 000	*WRN*	Skin cancer, soft-tissue sarcoma	N/A	[89,90]
Wilms tumor	Sporadic or hereditary	1 in 10 000	*WT1*	Kidney cancer	N/A	[91]

[a]Abbreviations: GBM, Glioblastoma multiforme; iPSC, induced pluripotent stem cell; LFS, Li–Fraumeni syndrome; NPCs, neural progenitor cells; NS/JMML, Noonan syndrome/juvenile myelomonocytic leukemia; NSC, neural stem cells; PSC, pluripotent stem cell.

defective osteoblastic differentiation and tumorigenic ability. Gene expression in LFS osteoblasts is also similar to the expression profile in primary osteosarcomas, particularly the more aggressive phenotypes. LFS-derived osteoblasts are free of cytogenetic rearrangements, permitting study of early oncogenic mechanisms prior to accumulation of secondary genomic alterations. Expression of the long noncoding RNA H19 had been previously linked to p53 activity [36] and transcriptome analysis suggested impaired expression of H19 in LFS osteoblasts. Further functional studies showed that H19 is essential for normal osteogenesis and inhibition of tumorigenesis. The LFS iPSC disease model uncovered a previously unidentified role of p53 in osteogenic differentiation defects and tumorigenesis.

Noonan Syndrome

Noonan syndrome (NS) is an autosomal dominant disorder characterized by a wide spectrum of congenital heart abnormalities, short stature, facial dimorphism, and predisposition to hematological malignancies. A subset of NS patients will develop juvenile myelomonocytic leukemia (JMML), an aggressive myelodysplastic and myeloproliferative neoplasm [37]. Mulero-Navarro *et al.* [31] investigated the molecular mechanisms involved in NS-associated JMML harboring PTPN11 mutations using hematopoietic cells derived from NS/JMML patient-specific iPSCs. These hematopoietic cells recapitulated several JMML characteristics including hypersensitivity to granulocyte-macrophage colony-stimulating factor and increased myeloid population. Comparison of transcriptome profiles of controls and NS/JMML-derived $CD33^+$ myeloid cells confirmed dysregulation of extracellular signal-regulated kinase (ERK) and Janus kinase/signal transducers and activators of transcription signaling (JAK/STAT) and proliferation of NS/JMML $CD33^+$ myeloid cells. Expression levels of miR-15a and miR-223 were also elevated in these cells. Notably, dysregulation of miR-15a and miR-223 is commonly observed in mononuclear cells isolated from JMML patients harboring PTPN11 mutations. Using the NS/JMML iPSC model, Mulero-Navarro *et al.* [31] demonstrated that inhibition of these miRNAs could restore normal myelopoiesis, providing a novel therapeutic target for PTPN11-mutated JMML.

Myelodysplastic Syndrome

Myelodysplastic syndrome (MDS) is a bone marrow disorder that leads to defective hematopoiesis and a disposition to develop anemia, cytopenia, and leukemia. Sporadic loss of one copy of the long arm of chromosome 5 [del(5q)] and/or chromosome 7 [del(7q)] is a characteristic cytogenetic abnormality in MDS [38]. Kotini *et al.* [32] established del(7q) MDS iPSCs from patient hematopoietic stem cells with loss of chromosome 7q and iPSCs derived from normal fibroblast isogenic controls. iPSCs with del(7q) recapitulated the phenotype of impaired myeloid lineage differentiation seen in MDS. This defective differentiation potential could be reproduced by engineering hemizygosity of definite 7q segments in normal iPSCs and could be rescued by spontaneous acquisition of an extra chromosome 7. Through phenotype-rescue screening, Kotini *et al.* [32] identified HIPK2, ATP6V0E2, LUC7L2, and EZH2 as haploinsufficient genes involved in del(7q) MDS-associated hematopoietic defects.

Diffuse Intrinsic Pontine Gliomas

Diffuse intrinsic pontine gliomas (DIPGs) are rare highly aggressive pediatric brain tumors that arise from glial tissue. Somatic p.Lys27Met substitution in histone 3.3 (H3.3K27M) is commonly detected in patients with DIPGs and is associated with poor survival [39,40]. Funato *et al.* [33] engineered human ESC-derived neural progenitor cells (NPCs) with heterozygous H3.3K27M mutations. To mimic genetic alterations found in clinical samples of H3.3K27M-mutated DIPGs, ESC-derived NPCs were transduced with lentiviruses also carrying constitutively active PDGFRA (D842V) and p53 small hairpin RNA. In this NPC model, H3.3K27M expression synergized with PDGFRA activation and p53 loss, culminating in neoplastic transformation. Genome-wide analyses of H3.3K27M-transformed NPCs revealed that they maintain both an undifferentiated epigenome structure and a primitive stem cell gene signature, enabling their tumorigenic potential. Using a small-molecule chemical library screen of compounds targeting epigenetic regulators, they identified the MEN1 inhibitor MI-2 as a potential drug for the subset of DIPGs harboring the H3.3K27M mutation. This study demonstrates the potential of PSCs for drug screening.

Glioblastoma Multiforme

Glioblastoma multiforme (GBM), also known as Grade IV astrocytoma, is a highly malignant brain tumor derived from glial cells. While GBMs are genetically very diverse, mutations in PTEN are common and correlate with increased invasion, drug resistance, and tumor recurrence [41]. Duan *et al.* [34] engineered PTEN-deficient ESCs using a TALEN-based genome-editing methodology and derived neural stem cells (NSCs) to model GBM. PTEN-deficient NSCs displayed the GBM-associated gene signature and formed intracranial tumors *in vivo*. Duan *et al.* [34] found that elevated levels of PAX7 contributed to neoplastic transformation by producing more aggressive phenotypes. Elevated PAX7 expression can be explained directly by PTEN deficiency since PTEN interacts with CREB/CBP and co-occupies the PAX7 promoter. Screening for anticancer compounds in PTEN-deficient NSCs suggested mitomycin C as a potential drug.

Directed Differentiation and Organoids

The potential of PSCs in modeling cancer is critically dependent on availability of defined methods to differentiate PSCs into the tissues from which tumors arise. The generation of specific cell or tissue types by directed differentiation and organoids is a fast-growing field in stem cell research [6–8].

Directed Differentiation

Directed differentiation is the application of a temporally defined set of external factors or culture conditions in order to produce a cell population enriched for a desired lineage. For example, we have employed a directed differentiation protocol to derive MSCs from p53-mutant iPSCs.

While the MSCs exhibited no obvious deficits and were free of chromosomal abnormalities, key oncogenic features representative of osteosarcoma emerged after their subsequent differentiation into osteoblasts [30].

PSC differentiation protocols have been established for generation of hepatic cells [42–44], intestinal tissue [45], thyroid follicular cells [46], airway epithelial cells [47–49], renal progenitor cells [50,51], retinal cells [52–55], epithelial stem cells [56], epidermal keratinocytes [57], myeloid cells [58,59], neurons [34,60], MSCs [61], and melanocytes [62], among many others. Application of these protocols to cancer patient-derived iPSCs and engineered ESCs should allow research of cancers of these organs.

Directed PSC differentiation protocols pose particular challenges to model cancer. PSCs can form teratomas, a rare tumor type from a normal genetic background. While differentiated cells derived from PSCs do not have this tumorigenic potential, even small contamination of desired differentiated cells with pluripotent cells can give the false appearance of tumorigenic properties. An effective differentiation protocol and purification scheme is therefore essential for any cancer study.

Despite these advances, there is a large unmet need for directed differentiation protocols for a broader set of cancer types, including breast, prostate, and ovary. As directed differentiation techniques from PSCs continue to develop, the versatility of using PSCs in cancer modeling will also expand.

Organoid Culture

Recent advances in 3D culture techniques combined with existing differentiation protocols have enabled the generation of PSC-derived specific tissue or progenitor cells within a self-organized assembly known as an organoid [7,8]. Compared with traditional 2D culture systems, these 3D organoid cultures mimic better their *in vivo* PSC-derived counterparts, hence positioning the technology as a powerful tool for studying human development and modeling disease.

3D cerebral organoids were derived by differentiation of human PSCs by Lancaster *et al* [63]. Matrigel droplets containing cerebral organoids were transferred into a spinning bioreactor, enabling a rapid, longer, and more abundant formation of 3D brain tissue. These 'mini-brain' systems facilitate the study of human brain development and have been used to model microcephaly, among other neurodevelopmental disorders. With the introduction of appropriate mutations, these brain organoid systems have the potential to enhance our understanding of brain tumor biology.

3D gastrointestinal organoids were also derived from PSCs by Wells *et al.* [45] and closely mimic the *in vivo* intestinal epithelium. These organoids contain crypt-like and villus-like structures and consist of differentiated enterocytes, goblet cells, and intestinal stem cells. Another protocol for generating PSC-derived gastric organoids [64] was later published by the same group. These 3D 'mini-stomach' systems were used to model *Helicobacter pylori* infection, shedding light on the pathogenesis of this common disease. Organoids generated from human intestinal stem cells were also used to recapitulate the colorectal adenoma-to-carcinoma transition by Matano *et al*. [65], highlighting the potential role of 3D organoid culture in studying gastrointestinal oncogenesis.

PSC-derived 3D culture and organoid systems have been used to model the liver bud [66,67], lung [47], and pancreas [68] from endoderm; the kidney [69–72] from mesoderm; and the optic cup [73–75] from ectoderm. These methodologies hold substantial potential for investigating the distinct tumor types derived from these now-accessible cell lineages.

Concluding Remarks

PSC disease models have not only succeeded in replicating disease phenotypes but have also begun to find applications in the understanding of disease biology and in the development of novel therapies (see Outstanding Questions). Applications of PSCs to cancer will be advantageous to (i) model disease phenotypes; (ii) elucidate pathological mechanisms; (iii) predict patient survival; (iv) identify potential biomarkers and therapeutic targets; (v) discover haploinsufficient genes by functional mapping of disease-associated chromosomal loss; and (vi) apply drug screening to identify potential compounds to rescue particular disease phenotypes.

The high levels of genomic alterations already present in cancer cell lines and tumor-derived mouse models make the elucidation of the initial steps of tumor development particularly challenging. Notably, iPSC-derived cells are free of cytogenetic rearrangements [4], allowing the study of early oncogenic mechanisms prior to the accumulation of secondary genomic alterations. A recent investigation comparing the mutational rate of somatic and pluripotent cell lines also demonstrated a tenfold lower mutation rate in iPSCs with each generation compared with somatic cells [76], highlighting the advantages of using genetically stable iPSCs rather than somatic or even more error-prone cancer cells to expand a cell population with a given genetic trait. PSC disease models should be useful in identifying and characterizing the 'second hit' during tumor development following a selective and stable introduction of the first one. Nonetheless, newly generated PSC lines should be fully characterized prior to experimental use to rule out chromosomal or genetic abnormalities.

Recent progress in genome-editing tools, including optimizations of the TALEN and CRISPR/Cas9 systems, made the generation of tailored alterations in PSCs from isogenic backgrounds feasible. These powerful tools enable cancer researchers to easily construct a particular model system to investigate the role of specific gene alterations in tumorigenesis in various tissues and organs (Figure 1B). While a number of genetic cancer syndromes exhibit incomplete penetrance, the incidence of disease and the risk factor of developing disease frequently cannot be accurately estimated due to the relatively small number of affected patients. Genome-manipulated ESCs from a wider range of genetic backgrounds may help complement studies from patient-derived iPSCs to clarify the role of genetic backgrounds in cancer predisposition from familial cancer syndromes.

Despite the versatility of PSC technology as a model for a number of cancer etiologies, the limited number of available protocols for tissue differentiation remains one of the major impediments precluding the wider application of this approach in cancer research. New developments in directed differentiation protocols and evolving 3D organoid culture techniques not only facilitate disease modeling in neuroscience, cardiology, and regenerative medicine – fields in which PSC models are currently frequently used – but also open up opportunities in cancer research as more tissue types become experimentally available. We hope that new PSC-derived organoid methodologies will fill in the gaps in our ability to produce many currently inaccessible cancer cell types, including breast, prostate, and ovary.

In conclusion, PSCs are destined for exciting future applications in the field of cancer biology. We look forward to the wide incorporation of PSC techniques into the toolkit of basic and clinical researchers seeking to efficiently recapitulate disease, characterize alterations stemming from specific cancer-associated mutations or hoping to complement their existing studies with a human model.

Outstanding Questions

Can the prognosis of and potential therapeutic strategies for cancers originating from differentiated lineages (e.g., osteoblasts, myelocytes, or astrocytes) be modeled reliably using patient induced pluripotent stem cell (iPSC)-derived cells?

Can the generation and manipulation of patient-derived iPSCs become sufficiently standardized to allow for the use of iPSC disease models in the clinical management of familial cancer syndromes?

Do iPSCs derived from distinct patients with the same cancer syndrome have similar gene expression profiles? Does individual genetic background significantly affect the gene expression profiles of distinct iPSC-derived tumors?

Can PSCs be applied to identify and characterize the 'second hit' during tumor development?

Are changes in patient-derived iPSCs equivalent to those in genome-manipulated ESCs in familial cancer syndromes?

Do *in vitro* reprogramming and differentiation processes trigger artificial tumor development in PSC disease models?

Can patient iPSC-derived cells be used as part of cancer immunotherapy to train the immune system to recognize a cancer signature?

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