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Review

Decoding cancer etiology with cellular reprogramming

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Cancer research remains clinically unmet in many areas due to limited access to patient samples and the lack of reliable model systems that truly reflect human cancer biology. The emergence of patient-derived induced pluripotent stem cells and engineered human pluripotent stem cells (hPSCs) has helped overcome these challenges, offering a versatile alternative platform for advancing cancer research. These hPSCs are already proving to be valuable models for studying specific cancer driver mutations, offering insights into cancer origins, pathogenesis, tumor heterogeneity, clonal evolution, and facilitating drug discovery and testing. This article reviews recent progress in utilizing hPSCs for clinically relevant cancer models and highlights efforts to deepen our understanding of fundamental cancer biology.

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Introduction

Since James Thomson's isolation and establishment of human embryonic stem cell lines [1] and Shinya Yamanaka's revolutionary development of induced pluripotent stem cells (iPSCs) [2,3], the scientific community has eagerly embraced these human developmentally relevant cell systems to study embryogenesis and disease etiology for nearly two decades. With the advent of cutting-edge genome editing technologies, including zinc-finger nucleases, transcription activator–like effector nucleases, clustered, regularly interspaced, short palindromic repeat/Cas9 (CRISPR/Cas9), and base editors [4,5], numerous experiments that were previously impossible due to the lack of available patient tissues are now being conducted on a large scale. By integrating defined lineage differentiation and organoid development [6], we are now able to differentiate these disease-relevant human pluripotent stem cells (hPSCs) into the cells of origin for many diseases, allowing us to model disease development, dissect disease mechanisms, and develop therapeutics for treatment [7].

Cancer is considered one of the most challenging diseases among human illnesses due to its complexity, widespread impact, and often devastating consequences. Unlike many other diseases, cancer involves the uncontrolled growth and spread of abnormal cells, which can invade and damage surrounding tissues and organs [8]. This makes it particularly challenging to treat. Given its complexity, resistance to treatment, and far-reaching impact on health and society, cancer remains a life-threatening disease that necessitates ongoing research, advanced therapies, and comprehensive prevention strategies.

For over 50 years, cancer researchers have employed cell lines, primary cells, patient-derived xenografts, samples, and small organism models, including fruit flies, zebrafish, and mice to understand cancer. Despite these efforts, the inherent complexity of the cancer genome and species-specific differences frequently hinder the effective translation of these discoveries into clinical applications. To overcome these drawbacks, many research groups, including ours, have employed human-based models to study cancer development, understand the underlying pathological mechanisms and treatment resistance, and aim to develop novel therapeutics for cancer patients. A series of pioneering studies using cancer-relevant iPSCs [9–13] and engineered hPSCs [14,15] in cancer research have enhanced our understanding of malignant cell states and the oncogenic transformations occurring at both the premalignant

initiation stage and the malignant late stage. Subsequently, various cancer-relevant patient iPSCs and engineered hPSCs were generated to study a range of cancer types, including sarcoma [9,10,16–18], retinoblastoma [19–21], myelodysplastic syndrome (MDS), leukemia [11,12,22–31], colorectal cancer [13,32], pancreatic cancer [33–35], lung cancer [36,37], ovarian cancer [38], kidney cancer [39], gliomas [14,15,40–43], medulloblastoma [44,45], atypical teratoid/rhabdoid tumor (ATRT) [46], and neuroblastoma [47] over the following years. These studies not only provide compelling evidence for the feasibility of using hPSC platforms in cancer research but also present an alternative model for integrating and comparing with other cancer model systems.

This review highlights recent studies using patient-derived iPSCs and engineered hPSCs to dissect cancer etiology, develop therapeutic testing, and identify promising treatments. Due to space limitations, we have focused on iPSC-related cancer studies that not only generate cancer models but also offer significant insights into cancer mechanisms and therapeutic development.

Nervous system tumors

The human nervous system functions as a comprehensive communication network throughout the body, consisting of the central nervous system (CNS), which contains the brain and the spinal cord, and the peripheral nervous system (PNS), which is made up of peripheral nerves originating from the CNS [48]. As the nervous system coordinates and controls all functions of the body, cancer of the nervous system is among the most prominent and malignant types of solid tumors. Various iPSC-derived platforms, including *in vitro* differentiated cell cultures and *in vivo* tumor models, have been developed to investigate the underlying molecular mechanisms and facilitate anticancer drug screening for CNS tumors, such as astrocytoma [40], diffuse intrinsic pontine glioma (DIPG) [41], low-grade glioma (LGG) [42], medulloblastoma [44,45], glioblastoma multiforme [43], and atypical teratoid/rhabdoid tumor [46], as well as PNS tumors like neuroblastoma [47].

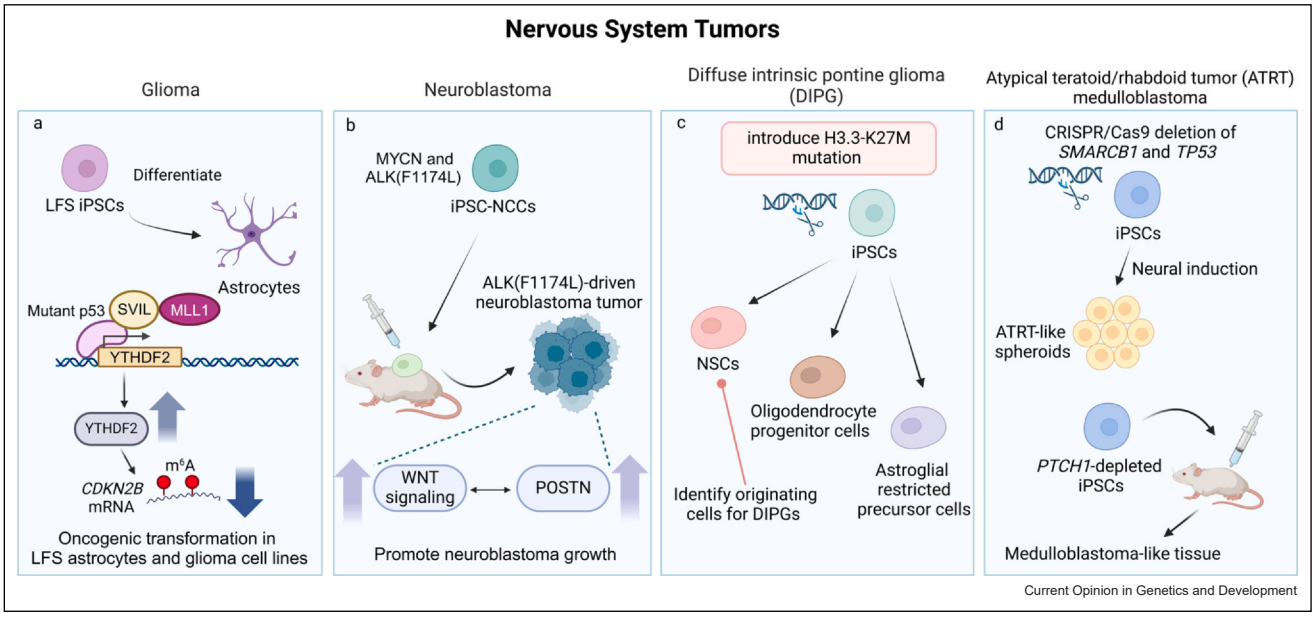
Glioma is the most common, devastating, and least curable malignant tumor of the CNS, originating from the glial cells of the brain and spine. Using iPSC-derived astrocytes from Li-Fraumeni syndrome (LFS) patients carrying an inherited germline p53(G245D) mutation [49], as well as those from healthy family controls transduced with various mutant p53s, Xu et al. identified a transcriptional complex formed by mutant p53, actin/myosin-II-binding protein SVIL, and H3K4me3 methyltransferase MLL1, which drives gliomagenesis [40]. The mutant p53/SVIL/MLL1 complex binds to the promoter of the N6-methyladenosine (m^6A) reader YTHDF2, transcriptionally activating its expression. The aberrant upregulation of YTHDF2 promotes the degradation of m^6A-marked *CDKN2B* and *SPOCK2* transcripts, leading to neoplastic transformation in LFS-derived p53-mutant astrocytes. These findings shed light on the molecular events underlying early gliomagenesis in patients with LFS and suggest potential therapeutic strategies for preventing and treating p53-mutant gliomas by targeting the mutant p53/SVIL/MLL1 complex, as well as its downstream effector YTHDF2 (Figure 1a).

Neuroblastoma is a pediatric tumor originating from neural crest cells (NCCs). To investigate the pathological mechanisms triggering neuroblastoma, Huang et al. developed transgenic iPSC-derived NCCs that stably expressed MYCN and/or a constitutively active ALK(F1174L) mutant [47]. While forced expression of MYCN in iPSC-NCCs led to tumors resembling neuroblastoma, ALK (F1174L) alone was insufficient to initiate neuroblastoma. However, ALK(F1174L) can work in conjunction with MYCN to drive neuroblastoma pathogenesis and further enhance tumor malignancy. Mechanistically, a regulatory circuit linking POSTN and WNT signaling operates downstream of ALK(F1174L) to promote cancer cell adhesion, migration, and growth. Thus, targeting POSTN and WNT signaling may offer potential therapeutic approaches for ALK-driven tumors (Figure 1b).

Elucidating the cell-of-origin of cancers is a fundamental aspect of cancer biology. To determine the originating cells for DIPG driven by the H3.3-K27M mutation, Haag et al. used CRISPR/Cas9 to introduce a conditional H3.3-K27M mutation and differentiated iPSCs into various neural progenitor cells, including neural stem cells (NSCs), oligodendrocyte progenitor cells, and astroglial-restricted precursor cells [41]. They demonstrated that H3.3-K27M NSCs, but not glia progenitors, are the originating cells for H3.3-K27M-driven DIPGs, supporting previous Funato K's study [15] (Figure 1c). Another study performed by Anastasaki et al. leveraged the versatility of the iPSC platform and advances in CRISPR/Cas9 technology to study potential originating cells for LGGs [42]. They discovered that neuroglial progenitor populations, including neural progenitors, glial restricted progenitors, and oligodendrocyte progenitors, could be involved in LGG formation, while terminally differentiated astrocytes are not. Furthermore, co-deleting *SMARCB1* and *TP53* genes in iPSCs, followed by neural induction, leads to the formation of ATRT-like spheroids [46], and subcutaneous injection of iPSCs with PTCH1 knockdown into immunodeficient mice results in the development of medulloblastoma-like tissue [45] (Figure 1d).

Taken together, these findings highlight the diverse applications of iPSCs in modeling and exploring the

Figure 1



Application of hPSCs for understanding the etiology of nervous system tumors. **(a)** iPSCs derived from an LFS family carrying a p53 mutation were differentiated into astrocytes. A transcriptional complex composed of mutant p53, SVIL, and MLL1 binds to the promoter region of *YTHDF2*, leading to its transcriptional activation and subsequent degradation of *CDKN2B* mRNAs. This process ultimately drives oncogenic transformation in LFS astrocytes. Additionally, the gain-of-function of this mutant p53 contributes to glioma progression in glioma cell lines. **(b)** NCCs derived from iPSCs that stably express MYCN and ALK(F1174L) mutations initiate neuroblastoma. In these ALK-driven neuroblastoma tumors, a regulatory circuit linking WNT signaling with POSTN promotes tumor growth. **(c)** Characterization of the cell-of-origin of H3.3-K27M-mutated DIPGs suggests that these tumors arise from NSCs rather than oligodendrocyte progenitor cells or astroglial-restricted precursor cells. **(d)** Normal iPSCs are engineered with deletions of *SMARCB1* and *TP53* and then differentiated into neural spheroids, which exhibit an ATRT-like phenotype. Additionally, *PTCH1*-depleted iPSCs, when transplanted *in vivo*, develop medulloblastoma-like tissues. Created with Biorender.com.

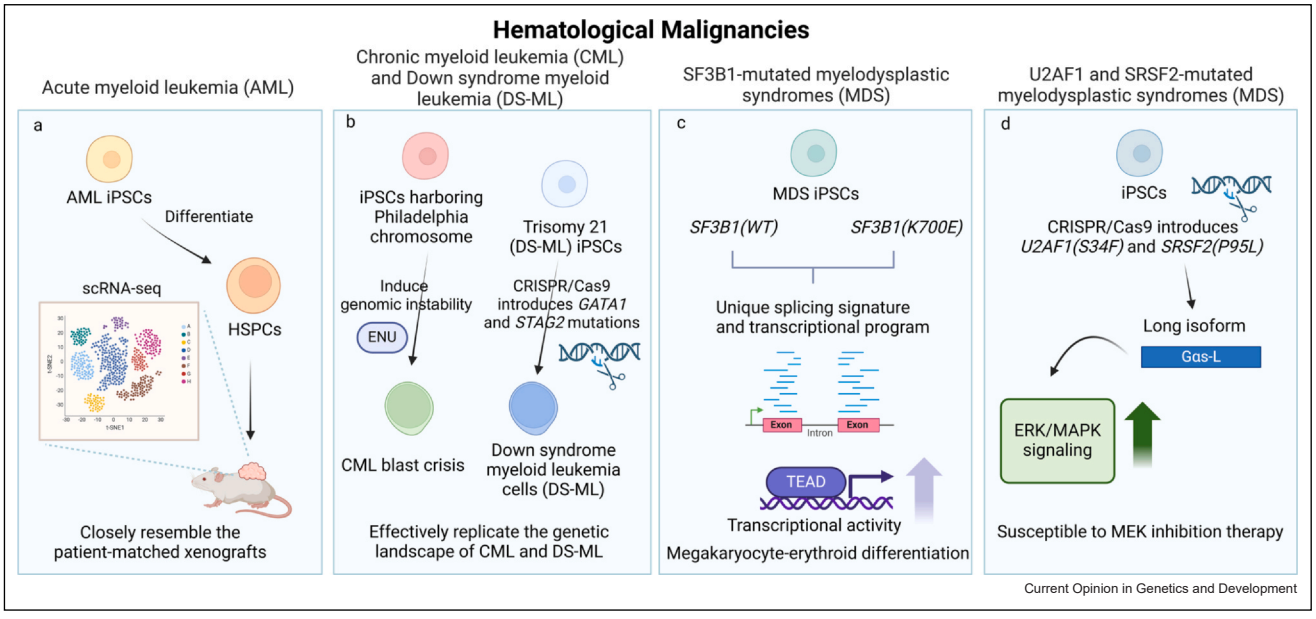
etiology and the cell-of-origin of nervous system cancers, as well as in anticancer drug testing.

Hematological malignancies

Hematological malignancies are a diverse group of blood cancers characterized by abnormal hematopoiesis and categorized into three major types: leukemia, lymphoma, and myeloma [50]. Among adults, acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) are the most prevalent forms of these malignancies. AML is an aggressive hematologic malignancy that arises from hematopoietic stem and progenitor cells (HSPCs), which are essential for producing blood cells in the bone marrow [51]. AML is characterized by the accumulation of immature myeloblasts in bone marrow, peripheral blood, or other tissues, disrupting normal hematopoiesis [51].

Kotini et al. successfully reprogrammed bone marrow mononuclear cells (BMMCs) and peripheral blood mononuclear cells isolated from 15 patients across major genetic AML subtypes into iPSCs (AML iPSCs), which were then differentiated into HSPCs [22]. The derived HSPCs displayed key leukemic features both *in vitro* and upon engrafted into immunodeficient mice. Importantly, single-cell transcriptomic analysis confirmed that the leukemias derived from these HSPCs closely resembled the primary patient-matched xenografts, highlighting their potential as robust models for studying AML (Figure 2a). In addition, Wang et al. presented another AML iPSC modeling approach by leveraging CRISPR/Cas9 technology to introduce heterozygous AML-associated mutations, including ASXL1 C-terminal truncation, SRSF2(P95L), and NRAS(G12D) in normal iPSCs [23]. The introduction of these three driver mutations into iPSC-derived HSPCs captured key aspects of primary MDS and AML, including phenotypic, transcriptomic, and chromatin features. In addition to AML, patient-derived iPSCs harboring the BCR::ABL1 Philadelphia chromosome have been used to model CML [24], a myeloproliferative neoplasm characterized by abnormal growth and overproduction of mostly myeloid cells at the expense of other blood cells in the bone marrow. These iPSCs were treated with the mutagenic agent N-ethyl-N-nitrosourea (ENU) to induce genomic instability, generating leukemic cells with genomic alterations commonly found in patients with CML during the blast crisis phase. Additionally, Down syndrome myeloid leukemia (DS-ML), a form of leukemia associated with Down syndrome and mutations in

Figure 2



Utilizing hPSCs to investigate the etiology of hematological malignancies. **(a)** iPSCs derived from patients with AML are differentiated into HSPCs and used in xenograft models. Single-cell transcriptomics reveals that the resulting tumors closely resemble those found in patients with AML. **(b)** iPSCs derived from patients with CML are treated with ENU to induce genomic instability, leading to the formation of leukemic cells, recapitulating CML blast crisis. Furthermore, introducing GATA1 and STAG2 mutations into trisomy 21 iPSCs results in the development of DS-ML. **(c)** iPSCs derived from patients with MDS are used to study the SF3B1(K700E) mutation. SF3B1(K700E) HSPCs display a distinct splicing signature and transcriptional program compared to wild-type counterparts, including increased activity of the TEAD transcription factor family. **(d)** Normal iPSCs are engineered to harbor U2AF1(S34F) and SRSF2(P95L) mutations, which result in the accumulation of Gαs-L and subsequent activation of the ERK/MAPK signaling pathway. This genetic modification renders the cells susceptible to MEK inhibition therapy. Created with Biorender.com.

the hematopoietic transcription factor GATA1 gene, was modeled using patient-derived iPSCs. CRISPR/Cas9 was employed to introduce GATA1 and STAG2 double mutations, effectively replicating the genetic landscape of DS-ML [25] (Figure 2b).

MDS are myeloid malignancies characterized by the expansion of a single dominant hematopoietic stem cell population, impaired blood cell production, and an elevated risk of progression to secondary AML [26]. Mutations in splicing factors, such as SRSF2, U2AF1, and SF3B1, are the most prevalent mutations in MDS [52]. Among these, mutations in the core RNA splicing factor SF3B1, present in approximately 30% of MDS cases, are the hallmark of the MDS-RS (MDS with ring sideroblasts) clinical subgroup [27]. In a study by Asimomitis et al., 18 iPSC lines were generated from BMMCs of three patients with MDS-RS carrying isolated SF3B1(K700E) mutation, the most frequent mutation in MDS, and their genetically matched SF3B1(WT) iPSCs to explore MDS-RS pathogenesis. Analysis of the transcriptomic and chromatin landscape in these iPSC-derived HSPCs revealed a unique splicing signature and transcriptional program linked to the SF3B1(K700E) mutation. Furthermore, their study showed that SF3B1(K700E) HSPCs exhibited an enhanced propensity for megakaryocyte-erythroid differentiation and a Hippo pathway-independent increase in the transcriptional activity of the TEAD transcription factor family, suggesting that TEAD could be a potential therapeutic target specific to SF3B1(K700E)-mutated MDS-RS (Figure 2c). In addition, CRISPR/Cas9-engineered U2AF1(S34F) and SRSF2(P95L)-mutated iPSC-derived HSPCs were also employed to investigate the oncogenic roles of these heterozygous splicing factor mutations [28]. The study demonstrated that these mutations resulted in alternative splicing, producing a long isoform of GNAS (GNAS-L), which is implicated as a phenotypic driver of MDS. Notably, GNAS-L encodes Gαs-L, a hyperactive long form of the stimulatory G protein alpha subunit Gαs, which activates ERK/MAPK signaling, promoting MDS development and rendering U2AF1- and SRSF2-mutant cells particularly susceptible to MEK inhibition therapy (Figure 2d).

Interestingly, chromosomal translocation is a prominent genomic abnormality found in blood diseases. Nakamura et al. generated iPSC lines from patients with MDS harboring the t(3;8)(q26.2;q24) translocation and investigated the resulting iPSC-derived hematopoietic

progenitor cells [29]. These cells successfully replicated the disease phenotype by demonstrating MECOM upregulation through increased H3K27ac and activation of the MYC blood enhancer cluster. Using this model, they discovered potential therapeutics by treating MDS cells with the BET inhibitor JQ1, which suppressed super-enhancer activity and downregulated MECOM expression, highlighting the potential of iPSCs as a powerful platform for discovering and evaluating therapeutic targets and drugs for MDS treatment.

Fanconi anemia (FA), a prototypical inherited bone marrow failure syndrome, has a high risk of progressing to MDS and AML. This transformation often involves HSPCs acquiring abnormal self-renewal capabilities due to somatic mutations. Marion et al. employed screening of MDS hotspot mutations and found that RUNX1 mutations drive aberrant HSPC self-renewal and impaired differentiation [30]. Mechanistically, FA MDS iPSC-derived HSPCs exhibited mutant RUNX1-mediated disruption of the G1/S cell cycle checkpoint and activation of innate immune signaling, which stabilizes BRCA1 and suggests targeted treatment options. Their results provide insight into MDS pathogenesis and offer a platform for discovering new therapies for FA-associated MDS.

Retinoblastoma

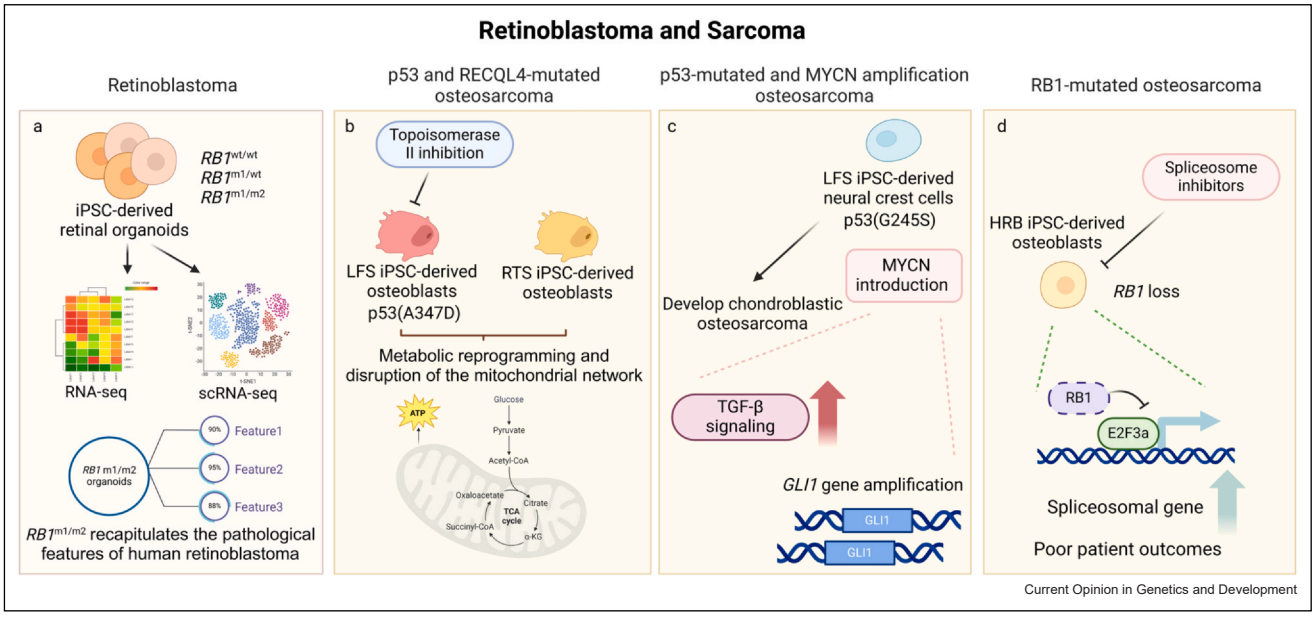
Retinoblastoma is a rare cancer that affects children, typically arising in the retina during fetal development and being diagnosed within the early years of life [53]. In most cases, retinoblastomas are developed due to biallelic mutation of the *RB1* tumor suppressor gene. Patients with a germline mutation in one *RB1* allele are at a high risk of developing retinoblastoma, with tumors forming when the remaining *RB1* allele becomes mutated, which is well-known as Knudson's 'two-hit' hypothesis [54]. Norrie et al. developed hereditary retinoblastoma (HRB) iPSC-derived retinal organoids from 15 individuals with germline *RB1* mutations and from CRISPR/Cas9-engineered counterparts, where specific mutations were introduced to inactivate RB1 [19]. These organoids were orthotopically transplanted into the vitreous of mice to examine retinoblastoma formation *in vivo*. Their study showed that these iPSC-based organoid-derived retinoblastomas faithfully recapitulated the molecular, cellular, histopathologic, genetic, epigenetic, and clonal characteristics observed in patient retinoblastomas and orthotopic patient-derived xenografts.

Given the advantages of organoids in modeling retinoblastoma and the critical role of biallelic *RB1* mutations in retinoblastomagenesis, Rozanska et al. developed organoid models derived from iPSCs generated from a patient with a heterozygous *RB1* mutation and their CRISPR/Cas9-engineered counterparts, including homozygous mutants and corrected isogenic controls [21]. Their study demonstrated that RB1 homozygous mutant, but not wild-type or heterozygous mutant, iPSC-derived organoids exhibited high mitotic activity, tumorigenic signatures, and excessive proliferation of cone precursors, identified as retinoblastoma-like clusters through single-cell RNA-seq analysis. Additionally, the RB1 homozygous mutant organoids were effectively used for antiretinoblastoma drug testing, highlighting the potential of this iPSC-derived model for drug development. In line with Rozanska A's study, Li et al. utilized heterozygous frameshift germline RB1-mutated 'first-hit' HRB iPSCs and introduced a 'second hit' mutation via CRISPR/Cas9-mediated gene knock-in to create compound heterozygous mutations, thereby validating Knudson's 'two-hit' hypothesis [20]. Wild-type, heterozygous HRB, and compound heterozygous HRB iPSCs were differentiated into human retinal organoids and subjected to RNA-seq analyses to identify differentially expressed genes. Tumor formation and pathological features of human retinoblastomas were observed in the compound heterozygous HRB organoids, but not in wild-type or heterozygous HRB organoids (Figure 3a). These findings introduced novel iPSC-derived models for studying retinoblastoma and provided strong evidence supporting Knudson's theory.

Sarcoma

Osteosarcoma is one of the most common primary bone malignancies in childhood and adolescence. It occurs in patients with several cancer-prone genetic disorders, such as LFS, HRB, and Rothmund-Thomson syndrome (RTS) [55]. Germline mutations in the *p53*, *RB1*, and *RECQL4* genes, which lead to inherited cancer syndromes, are also frequently observed as somatic mutations in sporadic osteosarcoma specimens, underscoring the critical role of these genes in osteosarcomagenesis. Choe et al. investigated the p53(A347D) oligomerization mutation identified in patients with LFS [56]. Using LFS iPSC-derived osteoblasts, which serve as a cellular model for osteosarcoma, and CRISPR/Cas9-engineered osteosarcoma cell lines, they discovered that p53(A347D) exhibits tumorigenic properties similar to p53-null cells. However, p53(A347D) also displays unique gain-of-function activities, such as metabolic reprogramming and disruption of the mitochondrial network. Additionally, the mutant enhances apoptosis in response to topoisomerase II inhibition in a transcription-independent manner. These findings suggest that p53(A347D) possesses both loss-of-function and neomorphic gain-of-function characteristics, presenting novel therapeutic opportunities using topoisomerase II inhibitor etoposide to treat p53-mutant osteosarcoma. Interestingly, dysregulation of mitochondrial function is also observed in RTS iPSC-derived osteoblasts [17], highlighting the critical role of

Figure 3



Dissecting the etiology of retinoblastoma and sarcoma via hPSCs. **(a)** iPSC-derived retinal organoids carry three different *RB1* genotypes (wt/wt, m1/wt, and m1/m2). Transcriptomic analysis showed that only the *RB1*(m1/m2) genotype recapitulates the pathological features of human retinoblastoma. m, mutation; wt, wild-type. **(b)** iPSCs derived from patients with LFS with p53(A347D) mutation and patients with RTS with RECQL4 mutations, when differentiated into osteoblasts, exhibit similar phenotypes, including metabolic reprogramming and disruption of the mitochondrial network. Topoisomerase II inhibitor etoposide can be used to treat cancers with the p53(A347D) mutation. **(c)** LFS patient-derived iPSCs carrying the p53(G245S) mutation develop chondroblastic osteosarcoma upon MYCN introduction, which activates TGF-β signaling and amplifies *GLI1* gene. **(d)** HRB patient-derived iPSCs with RB1 mutation are differentiated into osteoblasts, revealing that RB1 acts as a transcriptional repressor of E2F3a-regulated spliceosomal genes. Upregulation of spliceosomal genes in RB1-deficient osteoblasts correlates with poor patient outcomes and higher sensitivity to spliceosome inhibitors. Created with Biorender.com.

mitochondrial homeostasis in preventing abnormal cellular transformation in bone tissue (Figure 3b). Consistent with this finding, the LFS iPSC model with the p53(G245D) hotspot mutation was effectively used to investigate mutant p53's gain-of-function and its associated tumorigenic properties, including impairing the H19 imprinted gene network [9] and promoting tumor angiogenesis by upregulating SFRP2 expression [10]. In addition, by introducing MYCN into LFS iPSC-derived NCCs with a p53(G245S) hotspot mutation, Mukae et al. generated transformed clones that were used to develop chondroblastic osteosarcoma *in vivo* [57]. Gene expression analysis and exome sequencing revealed osteosarcoma-specific features, such as activation of transforming growth factor (TGF)-β signaling and amplification of the *GLI1* gene (Figure 3c). These findings underscore the model's value as a tool for studying MYCN-overexpressing and p53-mutated chondroblastic osteosarcoma and for developing new treatments for this type of cancer.

Another study presented by Tu et al. developed HRB patient-derived iPSCs and mutation-corrected isogenic controls to explore how *RB1* mutations drive tumor initiation and progression, with a focus on osteosarcoma [16]. They demonstrated that RB1 regulates spliceosomal gene expression through its role as a transcriptional repressor, inhibiting the transcriptional activator E2F3a. Analysis of clinical samples and large-scale data confirms the importance of the RB1/E2F3a network across various cancers, highlighting a connection between high spliceosomal gene expression and poor patient outcomes. The research also identifies the spliceosome as a critical vulnerability in RB1-mutant cancers, suggesting that targeting spliceosome function could be an effective therapeutic strategy (Figure 3d). Current trials of spliceosome inhibitors show promise in cancer treatment, indicating that this approach could be valuable for treating RB1-related osteosarcoma as well as other cancers with *RB1* mutations.

Interestingly, while mutations in p53, RB1, and RECQL4 all lead to osteosarcomagenesis, mechanistic studies using iPSC approaches reveal diverse pathological mechanisms with some overlapping hallmarks of cancer, such as mitochondrial dysfunction. This indicates that the oncogenic signals leading to osteosarcoma are complex and involve multiple coordinated pathways, rather than a single unique signaling pathway.

Mesenchymal chondrosarcoma is a rare malignant soft-tissue tumor, and gene fusions resulting from

chromosome rearrangements have been recognized as driver mutations in this cancer. The HEY1–NCOA2 gene fusion, a recurrent chromosomal rearrangement, has been identified as a key driver mutation. Qi et al. investigated mesenchymal chondrosarcoma by stably transducing an inducible HEY1–NCOA2 fusion gene into wild-type iPSC-derived mesenchymal stem cells, which are the cell-of-origin of mesenchymal chondrosarcoma, to study its effects [18]. Their comprehensive analysis revealed that the HEY1–NCOA2 fusion protein preferentially binds to promoter regions of HEY1 targets, enhances cell proliferation, and directly upregulates PDGFB and PDGFRA, leading to significantly increased AKT activity. These insights from the iPSC model suggest that targeting the PDGF/PI3K/AKT pathway could be a promising therapeutic approach for treating mesenchymal chondrosarcoma.

Lung cancer

RET oncogene rearrangements occur in a small percentage of lung adenocarcinoma. Marcoux et al. developed lung progenitor cells (LPCs) from patient-derived iPSCs carrying the RET(C634Y) mutation, analyzed the resulting abnormal cancer gene expression, and identified oncogenic markers PROM2 and C1QTNF6, both associated with poor non–small cell lung adenocarcinoma (NSCLC) outcomes [36]. The LPCs responded positively to pralsetinib, a RET inhibitor, highlighting the RET(C634Y) iPSC model's effectiveness in replicating NSCLC biology and its potential for therapeutic development. In parallel, tumor-like structures, morphological irregularity, proliferation capacity, and HER2/ESRRB-activated RAS/RAF/MAPK and PI3K/AKT/mTOR were discovered in HER2-overexpressing hiPSC-derived lung organoids [37], which reflects clinical lung cancer with HER2 amplification. These studies support that the iPSC model is capable of recapitulating the early tumorigenesis of lung cancers.

Colorectal cancer

Patients with constitutional mismatch repair deficiency (CMMRD) experience a complete loss of mismatch repair, leading to the development of colorectal cancer. Forster et al. investigated CMMRD in patients with a homozygous deletion of the pathogenic *EPCAM* gene, focusing on the occurrence of genome mutations in EPCAM-deleted iPSC-derived colonic organoids. They found MSH2 promoter hypermethylation and loss of MSH2, which resulted in a high mutation rate and characteristic features of mismatch repair deficiency [32]. In alignment with the use of colonic organoids derived from the familial adenomatous polyposis iPSCs to investigate colorectal cancer [13], these findings highlight the potential of iPSC and organoid models to reveal tissue-specific mechanisms underlying colorectal malignancies.

Future perspectives

iPSC technology has been employed in cancer research for over a decade, providing a unique platform to study the entire transformation process from normal to cancerous cells and uncover the underlying pathological mechanisms. Two primary hPSC-based cancer model systems — patient-derived iPSCs and engineered hPSCs — offer unique advantages in uncovering the oncogenic roles of oncogenes and tumor suppressor genes during the early stages of tumorigenesis, a crucial yet underexplored area in cancer research that holds significant potential for advancing cancer prevention. These models also support hypothesis-driven drug testing and the development of effective and clinically applicable treatments. With advancements in genome editing technologies and efficient gene delivery systems, hPSCs can now be readily manipulated to mimic cancer-associated mutations, deletions, fusions, and amplifications. By integrating organoid models and single-cell transcriptomics, we can analyze cancer heterogeneity, identify tumor-initiating cell populations, track clonal evolution, and even explore oncogenic signatures at the single-cell level.

The use of iPSCs in cancer modeling offers promising alternative approaches, providing a renewable and cancer-relevant cell source for investigating cancer biology. However, like any model system, there are several limitations and challenges that must be considered when using iPSCs to study cancer. These issues can significantly impact the insights gained from such models, and some key disadvantages include the following:

Snapshot of tumorigenesis and mutational representation: One of the major drawbacks of the iPSCs is that they may represent a snapshot of cancer at the initiation stage, potentially missing key features of cancer progression and evolution. Cancer is a dynamic disease, and its progression involves multiple genetic alterations that accumulate over time. When iPSCs are generated, they may not capture the full spectrum of driver mutations that evolve during the cancer progression. Specifically, the iPSC clones derived may reflect only a subset of the mutations present at the time of sampling rather than the entire clonal landscape of the patient's cancer. This is particularly relevant in cancers with chronic phases, where the disease evolves over time, with additional driver mutations accumulating to promote the evolution of cancer clones. As a result, iPSCs derived from a single gene alteration may only represent the initiation stage of tumorigenesis and may not adequately capture the complexity and heterogeneity of cancer across different stages. To make this iPSC cancer platform more applicable, incorporating additional driver mutations would provide a more comprehensive understanding of tumorigenesis and clonal evolution. For instance, studies

using hPSCs with multiple co-existing genome alterations will be essential for dissecting the various cancer subtypes (e.g. in glioma: LGG, diffuse LGG, anaplastic glioma, and glioblastoma) and their roles at different stages (e.g. in colorectal cancer: hyperplastic epithelium, adenoma, advanced adenoma with high-grade dysplasia, carcinoma, invasive carcinoma, and metastatic colorectal cancer). Moreover, iPSC reprogramming may preferentially select for or against certain clones, meaning that key mutations relevant to cancer progression might not be retained or may be challenging to reprogram.

Cancer's heterogeneity: Cancer is a heterogeneous disease with multiple clonal populations co-existing within a single tumor. This intratumoral heterogeneity is particularly pronounced in the later or chronic stages of cancer, where subclones may have distinct genetic and phenotypic features, contributing to disease progression, treatment resistance, and metastasis. iPSC models typically generate homogeneous cell populations, and thus, even though an iPSC line may represent a specific clonal population, it cannot capture the full complexity of the tumor's clonal architecture. To replicate the ongoing evolution of cancer, iPSCs can undergo repeated rounds of environmental stress, such as *in vivo* transplantation, to simulate the accumulation of driver mutations over time and model natural tumorigenesis. Additionally, introducing cancer-relevant mutations at different stages of differentiation can mimic clonal evolution, providing a more accurate representation of the heterogeneity observed in patient tumors.

Lack of tumor microenvironment: Another critical aspect of cancer evolution is the interplay between cancer cells and their microenvironment. Tumor cells continuously interact with various components of their niche, including stromal cells, immune cells, and extracellular matrix elements. These interactions play a key role in cancer progression, metastasis, and drug response. iPSC-derived cells, which serve as the origin of cancer cells and typically grow as homogeneous cultures *in vitro*, do not inherently replicate these complex cell–cell interactions. Therefore, modeling these interactions may require complex multicell-type co-cultured assembloids or organoids that incorporate multiple cell types to better reflect the *in vivo* niche and capture the broader context of cancer's evolutionary dynamics.

In conclusion, the future of cell reprogramming technologies in cancer research holds great promise. We anticipate that the insights gained from the ongoing integration of diverse areas in cancer and regenerative biology will deepen our understanding of tumor development and ultimately pave the way for new strategies in cancer prevention, as well as personalized treatments for those affected by cancers.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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