Systematic Review/Meta-analysis

ANNALS OF MEDICINE & SURGERY

OPEN

Brain tumors and induced pluripotent stem cell technology: a systematic review of the literature

Monir Shayestehfar, PhD[a], Tina Taherkhani, MD[a,*], Pardis Jahandideh, BSc[a], Amir A. Hamidieh, PhD[b], Mahsa Faramarzpour, MD[c], Amirhossein Memari, MD, PhD[a]

Background: Induced pluripotent stem cells (iPSCs) provide a novel approach to studying the pathophysiology of brain tumors and assessing various therapeutic techniques with greater precision. This study aims to systematically review the existing literature to critically analyze and synthesize current research findings. The objective is to evaluate the role of iPSCs in understanding brain tumors and in the development of innovative treatment strategies.

Methods: We systematically reviewed existing articles that utilized iPSC technology to assess either the pathophysiology of brain tumors or therapeutic techniques, following the standards of Preferred Reporting Items for Systematic review and Meta-Analysis guidelines. Key terms were comprehensively searched in electronic databases, including PubMed, EMBASE, and Scopus. Articles were screened based on specific inclusion and exclusion criteria. Ultimately, 22 relevant articles were chosen, and their data were extracted.

Results: The summary of findings for each selected article was organized into two general categories: "Methods of Generating iPSCs" and "Applications of iPSCs." The methods of iPSC generation, including transfection and transduction, as well as the types of viral or non-viral vectors used, were extracted and reported for each study. Additionally, the main aims of the selected studies, whether modeling or therapeutic approaches, were gathered and reported in the results section.

Conclusion: iPSC technology is a novel vehicle that brings new solutions to overcome difficulties in brain tumor studies. In vivo and in vitro models generated from iPSCs provide suitable platforms to investigate the pathophysiology of brain tumors more precisely. Also, iPSCs have been utilized in various studies to examine how different antitumor agents may affect the target cells.

Keywords: brain tumor, drug discovery, induced pluripotent stem cell, iPSCs, modeling, organoids

Introduction

Brain tumors exist in different types such as benign vs malignant and primary vs metastatic tumors. Despite the discoveries in multimodal therapies and a deeper understanding of tumorigenic processes throughout recent years, primary malignant brain tumors are among the most fatal and devastating forms of cancer[1]. Data showed that brain tumors are the second most common form of cancer leading to cancer-related morbidity and mortality in children[2]. Nevertheless, the research in this area is mainly limited by genetic heterogeneity and a lack of complete and efficient laboratory models. Moreover, the unique biology of the brain imposes a further degree of complexity in investigating pathological mechanisms of brain tumors and designing novel therapeutic strategies[2].

In this vein, numerous animal brain tumor models have been generated so far including carcinogen-induced animal models, xenograft animal models, and genetically engineered mouse models (GEMMs)[3,4]. Such animal models have been widely used to investigate pathological mechanisms involved in tumorigenesis and tumor biology in a physiological context, as well as preclinical testing of novel therapeutic regimens[5]. However, most of these models have had serious limitations in fully recapitulating human brain tumor heterogeneity and testing preclinical medications[1,6]. Patient-derived xenografts (PDX) have been developed to overcome the limitations of animal models and represent human brain tumors more closely. Although PDXs retain patient mutational heterogeneity, due to being derived from tumor tissue, they cannot be utilized to investigate mechanisms of tumorigenesis[2]. In addition, cancer cell lines include a homogenous cell population lacking tumor genetic heterogeneity, and during in vitro culture, they gradually lose the original tumor phenotypes and genetic features[5].

To address such limitations, organoids, which are an emerging technology, represent a major advancement in understanding brain tumorigenesis and developing new therapeutic strategies. Organoids are typically driven from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). Thanks to the pioneering work of Takahashi and Yamanaka in 2006, iPSC technology has become one of the most efficient systems for disease modeling, drug discovery, and designing novel

[a]Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran, [b]Pediatric Cell and Gene Therapy Research Centre, Gene, Cell & Tissue Research Institute, Tehran University of Medical Science, Tehran, Iran and [c]Leishmaniasis Research Center, Kerman University of Medical Sciences, Kerman, Iran

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

*Corresponding author. Address: Sport Medicine Research Center, Neuroscience Institute, Tehran University of Medical Sciences, 1417613151, Jalal e al e Ahmad Highway, Tehran, Iran Tel.: +989128838972.
E-mail: taherkhanitina778@gmail.com (T. Taherkhani).

Annals of Medicine & Surgery (2025) 87:250–264

Received 10 July 2024; Accepted 5 November 2024

Published online 9 January 2025

http://dx.doi.org/10.1097/MS9.0000000000002760

therapeutic strategies for different complex disorders, particularly brain disorders[7,8]. In fact, iPSC-derived organoids and brain cells represent similar genetic data, structure, and function of the brain, and this makes iPSCs compelling alternatives to traditional models of brain disorders, especially brain tumors[9]. This capability improves disease modeling, enabling researchers to investigate tumor diversity, interactions within the brain's environment, and the early stages of tumor development. Furthermore, iPSCs support effective drug screening tailored to individual tumor responses, promoting personalized treatment strategies. Overall, iPSCs significantly enhance understanding of brain tumors and their potential therapies.

However, brain tumor modeling is still challenging due to the poor prognosis of brain cancers and complex brain tumor biology[9]. In the past decade, only a limited number of studies have utilized iPSC technology to investigate brain tumors, such as gliomas and medulloblastomas[10-12]. Despite some valuable insights, there is a notable gap in the literature regarding comprehensive reviews that integrate findings across various research areas, including disease modeling, regenerative therapy, and drug discovery. Furthermore, the variability in methodologies and approaches further complicates the development of standardized protocols. This study aims to systematically review the application of iPSC technology in brain tumor research to address these gaps and provide a clearer understanding of its potential. We hope our systematic review could better represent the strength of evidence and suggest novel directions for future research in the field.

Methods

The search strategy, study selection, data extraction, and analysis were conducted and reported according to the standards of the Preferred Reporting Items for Systematic review and Meta-Analysis guidelines (PRISMA) in this study[13]. Also, this study has been conducted and reported in accordance with the Assessing the Methodological Quality of Systematic Reviews guidelines to ensure methodological consistency and transparency throughout the systematic review process[14].

Eligibility criteria

Articles utilizing iPSCs to develop a cellular model of brain tumors or to investigate the effect of medications or other regenerative therapy on a specific tumor of the brain were selected and included in the systematic review. The inclusion criteria were as follows: (1) use of iPSCs as the technology of interest, (2) having brain tumors as their exposure of interest, and (3) being published in the English language. In addition, studies including human or animal subjects as their sample of interest were both eligible to be included in the current systematic review. Letters, reviews, presentations, or any reports without sufficient data were excluded from the study. Papers were also excluded if they used a sample other than brain tumors, did not utilize iPSCs, were non-empirical in nature, or were review studies.

Information source and search strategy

Electronic databases including PubMed, EMBASE, Web of Science, and Scopus were comprehensively searched in December 2022. The key terms and words administered in the search strategy were as follows: "induced pluripotent stem cell" OR "human induced pluripotent stem cell" OR iPSC OR hiPSC AND "brain tumor*" OR glioblastoma OR glioma OR astrocytoma OR ependymoma OR oligoastrocytomas OR oligodendrogliomas OR meningioma OR "acoustic neuromas" OR "pituitary adenomas" OR "medulloblastomas" OR "craniopharyngiomas" OR schwannomas OR "choroid plexus carcinoma" OR oligodendroglioma OR pineoblastoma OR "pituitary tumors." A further manual search of references in review articles was conducted to find the relevant articles.

Eligibility criteria and study selection of articles

After removing the duplicates, in the primary search, 323 articles were entered into the library. According to title and abstract checking, 263 papers were excluded and 60 papers were selected to be screened on their full text. In the final phase, 23 papers reached the criteria for entering the systematic review (Fig. 1). The review and screening of search results were independently conducted by two reviewers (T.T. and P.J.) to find studies that met the inclusion criteria and any disagreement resolved by a third reviewer (M.S.).

Data collection process and data items

Data extraction was performed for the following data from each article by two independent reviewers according to the standardized data extraction form. Publication details (including the first author's name, year of publication, and the country in which the study was conducted), study details (including study design, species of sample, number of samples, gender of samples, age of samples, type of brain tumor, and control group characteristics (if there is a control group)), and aim of study/result details were extracted from each selected article. A non-available or not-reported statement was assigned in cases in which supplementary methodological information was not provided either from the article or the corresponding author's contact. Disagreements between the reviewers on the extracted data were resolved by a third reviewer or consensus-based discussion.

The data from 23 selected studies were extracted for narrative synthesis, providing a summary of the collected data and a descriptive representation of the findings presented in the Results section (Table 1). We presented data in the form of a narrative synthesis since the perceived heterogeneity of the studies was considerable. Primarily, we summarized the methods of generating iPSCs. Further results were organized based on the main approach of each selected study including modeling or therapeutic approaches, as well as the general application of iPSC technology.

Risk of bias assessment

The risk of bias was also assessed by two independent reviewers. In order to analyze the methodological quality, we utilized two different assessment tools, which specifically assess the quality of in vivo and in vitro studies separately. For studies that incorporated both in vitro and in vivo components, we conducted quality assessments using both assessment tools. In order to assess the quality of in vivo studies, we utilized the Systematic Review Center for Laboratory Animal Experimentation encompassing 10 items that are associated with six types of bias. The

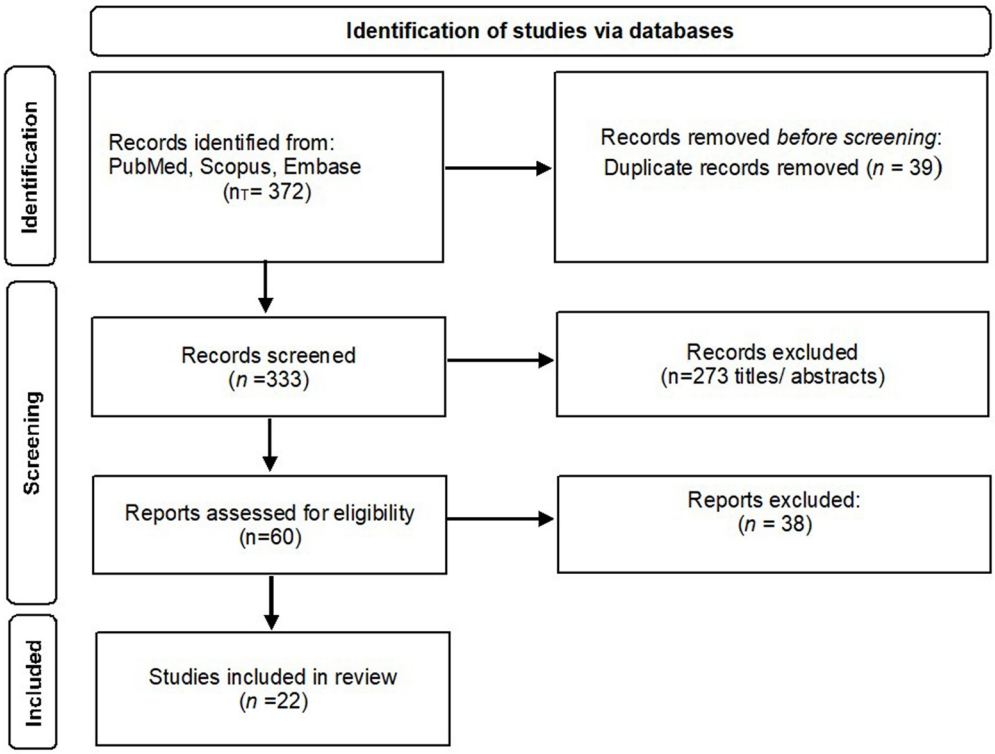

Figure 1. Identification of final selected studies based on the PRISM 2020 statement: an updated guideline for reporting systematic reviews.

items include selection bias (sequence generation, baseline characteristics, and allocation concealment), performance bias (random housing and blinding), detection bias (random outcome assessment and blinding), attrition bias (incomplete outcome data), reporting bias (selective outcome reporting), and other biases[15]. A "yes" was assigned for a low risk of bias, "no" for a high risk of bias, and "unclear" for no representing sufficient information. On the other hand, the quality of in vitro studies was evaluated using the Oral Health Assessment Tool, which is the National Health and Medical Research Council's recommendation for quality assessment of in vitro studies[16]. This assessment tool includes 11 risk of bias domains that address six types of bias: selection bias, confounding bias, performance bias, attrition/exclusion bias, detection bias, and selective reporting bias[17]. The risk of bias for each question was rated as follows: "+2" for definitely low, "+1" for probably low, "–1" for probably high, and "–2" for definitely high.

Synthesis method

We performed a qualitative synthesis of our data, as we were unable to pool the results together to perform a quantitative synthesis. We used tables and narrated the findings to summarize and synthesize the study's findings.

Results

Study selection and characteristics

Table 1 represents the details and characteristics of the final selected studies. In our primary search and after removing the duplicate studies, 333 articles were entered into the library in the first phase. In the second phase, two independent reviewers checked the articles for their titles/abstracts and removed 273 articles due to the lack of relevance of the subject. Furthermore, 60 articles remained for full-text checking by two independent reviewers, though 22 research articles met the inclusion criteria for being systematically reviewed in the current study. The main reasons for removing the articles were as follows: using a technology or tool other than iPSC technology, not assessing brain tumors, being non-empirical in nature, and being a review study or a conference presentation.

As Table 1 shows, the final articles were published in 11 different countries, with United States being the largest group with eight studies, followed by Japan with four studies. There was no clinical or randomized control trial in the selected studies. Of the final 22 studies, 19 research articles administered iPSC technology for modeling brain tumors, and the remaining three studies had interventional approaches toward brain tumors by administering iPSC technology. Two general categories were defined in order to check the species of animals that were used in the selected studies: (1) the primary cell source for iPSC generation and (2) the species type in which the iPSCs or brain tumor cells have been studied (Fig. 2). Thus, among the final selected studies, 21 studies administered human cells as the main source of generating iPSC/brain tumor and one study used non-human cells (mouse) for generating iPSC. In addition, regarding the second category, 14 studies used rodents including mice and rats as a hosts for iPSC/brain tumor cell transplantation. Moreover, 10 studies administered both in vivo and in vitro study designs, while the other remaining studies utilized a single

Table 1

Basic characteristics of final selected studies

	Author, year	Country	Study design/ sample	Brain tumor type	iPSC technology (transduction, transfection)/viral or non-viral/iPSC source	Host species (in case the study design is in vivo)	Type of virus/ reagent	Aim of study	Findings
1	Yamazoe *et al.*, 2014	Japan	Animal study/mouse/ in vivo and in vitro	Glioma	Transduction/mouse iPSC	Mouse xenograft	Retrovirus	Interventional/ mesenchymal stem cells	This study reports NSCs and MSCs as alternative therapeutic tools for malignant glioma Another report in this study is the safer iPSC-NSCs migratory activity, which were transduced from iPSCs after neural induction
2	Ignacio Sancho-Martinez *et al.*, 2016	USA	Animal study/mice/ in vivo and in vitro	Glioma	Transfection non-viral/ hiPSCs	Mice xenograft	Plasmid Lipofectamine	Interventional/Glioma tumor-initiating cells	This study surveyed iPSCs potentiality in tumorigenesis to investigate gliomagenesis
3	Bian *et al.*, 2018	China	In vitro	Glioblastoma	Transfection/hiPSC	Not applicable	NA	Modeling/Organoids	This study reports distinct transcriptional profiles, and different cellular identities for MYCOE and GBM-like neoCORs Results indicate that neoCORs remain viable and expand after renal subcapsular engrafting
4	Ogawa *et al.*, 2018	USA	Animal study/mouse/ in vivo and in vitro	Glioma	Transfection viral/ hiPSC	Mouse xenograft	CMV	Modeling/organoids	This study reports evidences that transformed cells rapidly become invasive and destroy surrounding organoid structures, overwhelming the entire organoid In addition, this study reports that human-organoid-derived tumor cell lines or primary human-patient-derived glioblastoma cell lines can be transplanted into human cerebral organoids to establish invasive tumor-like structure
5	Huang *et al.*, 2019	USA	Animal study/mice/ in vivo	Medulloblastoma	Transduction/hiPSC	Mice xenograft	Retrovirus and episomal plasmid	Modeling/ Medulloblastoma	This study showed that NES cells derived from Gorlin syndrome patients could generate medulloblastoma because of PTCH1 mutation
6	Liu *et al.*, 2019	UK	Animal study/mice/ in vivo and in vitro	LGGs	Transduction/hiPSC	Mice xenograft	Lentivirus	Modeling/Low-grade gliomas	This study recommends that regional chromosomal alterations may present prior to the acquisition of *IDH* mutations in at least some cases of LGGs
7	Plummer *et al.*, 2019	Scotland	In vitro	Glioblastoma	Transduction/hiPSC	Not applicable	Retrovirus	Interventional/ glioblastoma	This study introduced an approach to study anticancer medication response differences Moreover, TMZ and DOX treatments reasoned a reduction in the size of the gBS with little or no effect on the number of normal neuronal cells
8	Terada *et al.*, 2019	Japan	Animal study/mice/ in vivo and in vitro	Teratoid/Rhabdoid tumor	Transfection viral/ hiPSC	Mice xenograft	Sendai virus	Modeling/Neural progenitor-like cells	Findings in this study showed activation of the ESC-like signature in clinical specimens of AT/RTs but not medulloblastomas or glioblastomas

(*Continues*)

Table 1

(Continued).

	Author, year	Country	Study design/ sample	Brain tumor type	iPSC technology (transduction, transfection)/viral or non-viral/iPSC source	Host species (in case the study design is in vivo)	Type of virus/ reagent	Aim of study	Findings
									In addition, that c-MYC overexpression induces activation of the ESC-like signature in NPLC-derived tumors and drives tumor development with the rhabdoid phenotype
9	Ikemoto *et al.*, 2020	Japan	Animal study/mice/ in vivo and in vitro	Medulloblastoma/ Tratoma	Transfection viral/ hiPSC	Mice xenograft	Sendai virus	Modeling/ Medulloblastoma	This study used iPSC-derived from four Gorlin syndrome patient to clarify brain tumor cancers like basal cell carcinoma and medulloblastoma. There was not any correlation between Gorlin syndrome and GIn-iPSCs in non-medulloblastoma patients, but one of medulloblastomas demonstrated loss of PTCH1 gene
10	Koga *et al.*, 2020	USA	Animal study/mice/ in vivo	Glioblastoma	Transfection-non-viral/ hiPSC	Mice xenograft	Plasmid—lipofectamin	Modeling/ Glioblastoma	This study reports mesenchymal and proneural subtype features by NF1-deleted tumors and PDGF-driven tumors, respectively, in mouse models These cancer avatar models introduce a platform to assess human tumor development, governed by molecular subtype mutations and lineage-restricted differentiation
11	Tamura *et al.*, 2020	Japan	Animal study/mice/ in vivo	Glioblastoma	Transduction/hiPSC	Mice xenograft	Lentivirus	Interventional/Neural Stem/Progenitor Cells	This study reports that in the presence of a prodrug GCV, hiPSC-derived NS/PCs transduced with the lentiviral vector expressing HSV-TK were able to inhibit the growth of human glioma cells, through the bystander killing effect
12	Krieger *et al.*, 2020	Germany	In vitro	Glioblastoma	Transfection non-viral/ hiPSC	Not applicable	Plasmid—FuGene HD	Modeling/ Glioblastoma	This study reports recapitulating the in vivo behavior of GBM by showing an extended network of long microtubes in tumor cells with organoids
13	Haag *et al.*, 2021	USA	Animal study/mice/ in vivo	DIPG	Transfection non-viral/ hiPSC	Mice xenograft	Plasmid-FuGene HD transfection reagent (Promega)	Modeling/Astroglial and oligodendroglial differentiation	This study showed increase in neural stem cell proliferation, and a viability reduction in H3.3-K27M DIPG cells reported in overexpression of K27M specifically in H3.3 Also increase apoptosis and proliferation primarily in NSCs occurred by H3.3-K27M Viability decreases in iPSC, impairing pluripotency reprogramming, and effect on gene regulation with bivalent promoters reported by H3.3-K27M

(*Continues*)

Table 1

(Continued).

	Author, year	Country	Study design/ sample	Brain tumor type	iPSC technology (transduction, transfection)/viral or non-viral/iPSC source	Host species (in case the study design is in vivo)	Type of virus/ reagent	Aim of study	Findings
14	Anastasaki *et al.*, 2022	USA	Animal study/mice/In vivo and in vitro	Glioma	Transfection non-viral/ hiPSCs	Mice xenograft	NA	Interventional/LGGs	This study established a tractable experimental humanized platform for childhood brain tumors by elucidating the pathogenesis of and potential therapeutic opportunities
15	Baliña-Sánchez *et al.*, 2023	Spain	In vitro	Brain tumors	Transfection viral/ hiPSC	Not applicable	Sendai virus	Modeling/ mesenchymal stromal cells	This study introduces a non-invasive approach for brain tumor envisioned children's treatment by generating personalized iMSC products
16	Linkous *et al.*, 2019	USA	In vitro	Glioblastoma	NA/hiPSCs	Not applicable	NA	Modeling/ Glioblastoma	This study demonstrated glioblastoma invasion into organoids environment same as brain tissue and clarify its pathogenesis
17	Goranci-Buzhala *et al.*, 2020	Germany	In vitro	Glioblastoma	NA/hiPSCs	Not applicable	NA	Modeling/ Glioblastoma	This study showed that iPSC-derived organoids as a suitable platform to investigate medulloblastoma
18	Hwang *et al.*, 2020	France	In vitro	Glioblastoma	Transfection viral/ hiPSCs	Not applicable	Sendai virus	Modeling/ Glioblastoma	This study showed c-met mutated iPSCs generated glioblastoma related genes after 90 days in comparison with other iPSCs TMZ could be an efficient medication for c-met mutated iPSC-derived organoids
19	Cancer *et al.*, 2019	Sweden	In vitro	Medulloblastoma	Transduction/hiPSCs	Not applicable	Lentivirus	Modeling/ Medulloblastoma	This study benefited iPSC-derived organoids to investigate role of oct4 in activation of mTOR as a metastasis inducer in tumors
20	Susanto *et al.*, 2019	Sweden	Animal study/mouse/ in vitro and in vivo	Medulloblastoma	Transfection viral/ hiPSCs	Mouse xenograft	Sendai virus	Interventional/ Medulloblastoma	This study showed iPSC-derived human NES cells from a Gorlin syndrome patient which carrying a germline mutation in the sonic hedgehog receptor. PTCH1could mimic human medulloblastoma after implantation into mouse brain
21	Xue *et al.*, 2021	USA	Animal study/mice/In vitro and in vivo	Medulloblastoma	Transfection viral/ hiPSCs	Mice xenograft	Sendai virus	Modeling/ medulloblastoma	This study showed iPSC-derived medulloblastoma model utilized to evaluate cytotoxic effect of Frondoside A both in vitro and in vivo
22	Ballabio *et al.*, 2020	Italy	Animal study/mouse/ in vitro and in vivo	Medulloblastoma	Transfection non-viral/ hiPSCs	Mouse xenograft	Piggyback transposase	Modeling/ Medulloblastoma	This study investigated iPSC-derived organoids to evaluate role of Otx2 and cMYC in medulloblastoma formation

AT/RTs, teratoid/rhabdoid tumors; CMV, cytomegalovirus; DIPG, diffuse intrinsic pontine glioma; DOX, doxorubicin; ESC, embryonic stem cell; GBM, glioblastoma; gBS, glioblastoma brain sphere; GCV, ganciclovir; hiPSC, human induced pluripotent stem cell; HSV-TK, herpes simplex virus thymidine kinase; iMSC, induced mesenchymal stem cell; iPSC, induced pluripotent stem cell; LGG, low-grade glioma; MSCs, mesenchymal stem cells; mTOR, mammalian target of rapamycin, MYCOE, myc oncogene-expressing cells; neoCOR, neoplastic cerebral organoid; NES, neuroepithelial stem; NPLCs, neural progenitor-like cells; NS/PCs, neural stem/progenitor cells; NSCs, neural stem cells; TMZ, temozolomide.

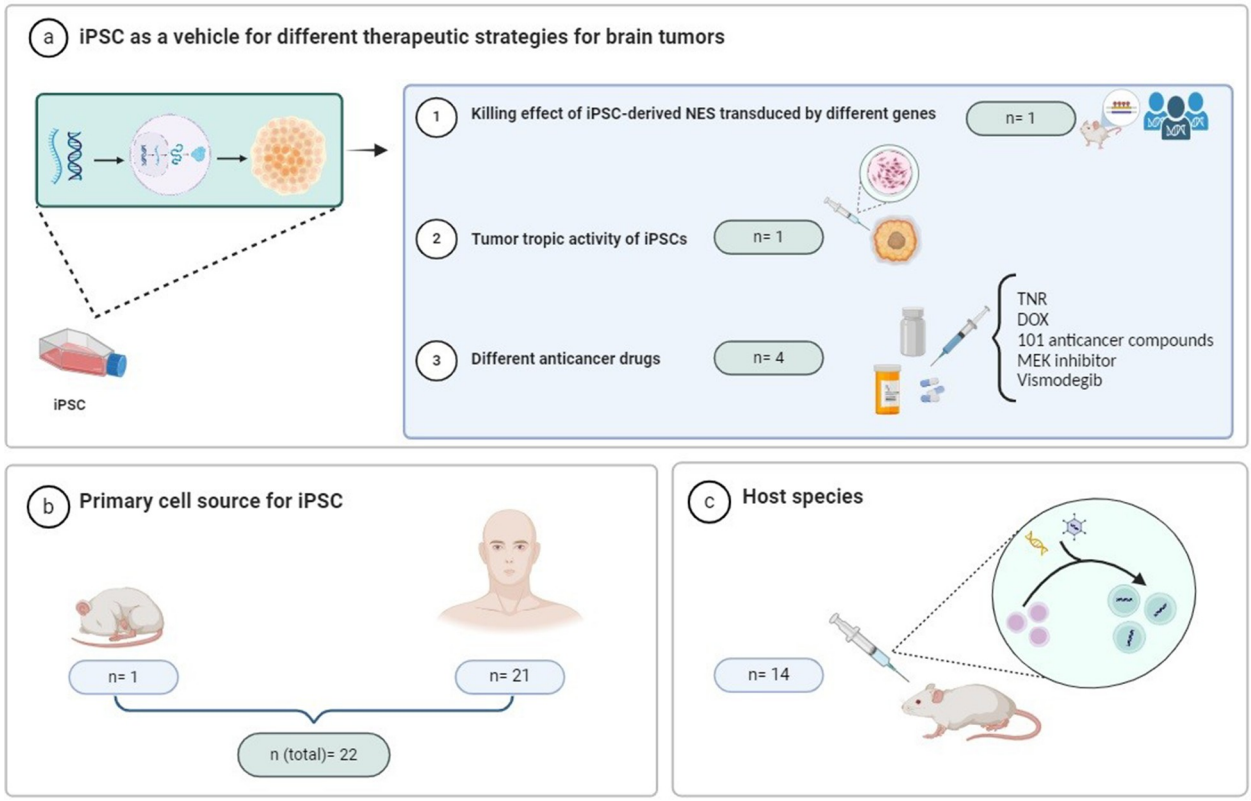

Figure 2. (A) iPSCs as a vehicle for therapeutic strategies and the number of selected studies in each category. (B) Primary cell source of iPSC generation. (C) Host species in selected in vivo studies. Illustrations used elements from BioRender.com (2021) (https://app.biorender.com/biorender-templates/, accessed on November 2023). iPSC, induced pluripotent stem cell.

study design, either in vivo (four studies) or in vitro (eight studies). In regard to the type of studied brain tumors, glioblastoma has been the most common brain tumor of interest with eight studies followed by medulloblastoma (six studies), glioma (four studies), diffuse intrinsic pontine glioma (one study), Tetartoid/Rhabdoid tumor (one study), and low-grade glioma (LGGs) (one study).

Summary of findings

Method of generating iPSC

Methods of generating iPSCs were surveyed in all 22 selected studies and categorized into two general types of transductions and transfection (Fig. 3). Six studies used transduction methods (viral/integrating), and 14 studies utilized transfection method (non-integrating), though two studies reported no data on the method of generating iPSCs. Moreover, the transfection method per se is divided into viral and non-viral methods. Three out of six transduction studies utilized lentivirus as a viral vector and the remaining two studies used retrovirus vehicles. On the other hand, seven out of 14 transfection studies utilized viral vectors including Sendai virus (six studies) and Cytomegalovirus (one study). In addition, seven out of 14 transfection studies investigated non-viral vectors including Fu gene HD (two studies)[10,18], lipofectamine (two studies)[12,19], and piggyback transposase (one study)[20]. The remaining two transfection studies reported no data on the type of utilized non-viral vectors.

Main iPSC applications

Generally, we categorized two different aims and approaches throughout the selected studies described in the following. It is worth mentioning that several studies had a mixed approach (i.e., both modeling and therapeutic approaches); however, we still categorized it based on the main approach of the article (Fig. 4).

iPSC in studies with therapeutic approaches

Six studies had therapeutic aims by administrating iPSC in both in vivo and in vitro settings (Fig. 4). For example, Tamura *et al.* assessed the effectiveness of iPSC-derived neural stem/progenitor cells, which were transduced by lentiviral viral vectors containing herpes simplex virus thymidine kinase (HSV-TK) suicide gene in orthotopic xenograft mouse model of human glioblastoma. The authors also showed that ganciclovir dramatically prevents glioblastoma cell growth in mice and significantly improves their survival compared to the control group and demonstrated the potential therapeutic benefit of binary hiPSC-derived neural stem/progenitor cells with herpes simplex virus-thymidine kinase/ganciclovir and ganciclovir[21]. Furthermore, Yamazoe *et al.* investigated the tumor tropic activity of both iPSC and iPSC-derived neural stem cells for C57BL/6 mouse glioma in an in vivo mouse intracranial tumor model, as well as in an in vitro Matrigel invasion chamber assay. They also observed the potent glioma tropism capacity of both iPSC and iPSC-derived neural

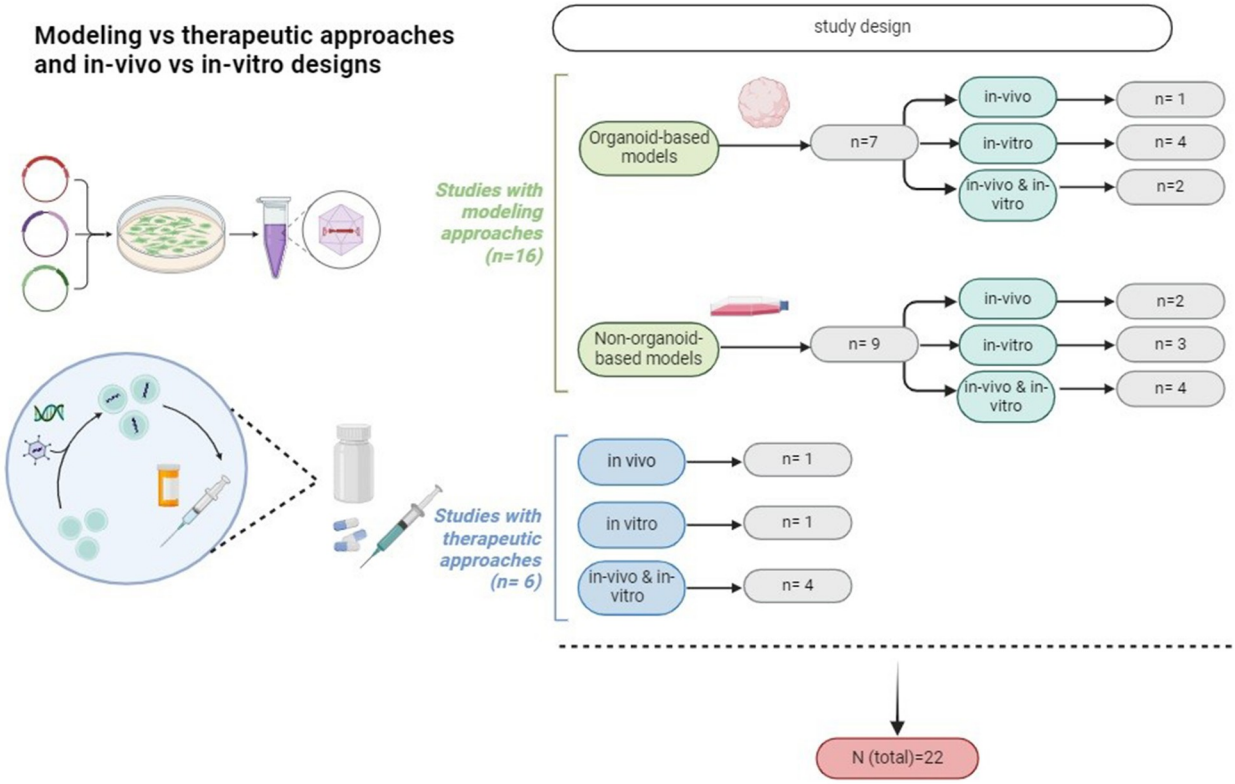

Figure 3. Method of generating iPSCs surveyed in all 23 selected studies. Among 23 selected studies, 14 studies administered transfection methods and seven studies used transduction methods. The details on the number of studies in each category are illustrated. Illustrations used elements from BioRender.com (2021) (https://app.biorender.com/biorender-templates/, accessed on November 2023). iPSC, induced pluripotent stem cell.

stem cells in mouse glioma[22]. In another investigation, Plummer *et al.* developed a human glioblastoma spheroid micro-physiological system called gBS, which contained iPSC-derived neuronal cells and primary glioblastoma cells. In this system, these two types of cells co-cultured together in a heterotypic spheroid model to investigate the effect of anticancer medications such as temozolomide (TMZ) and doxorubicin (DOX) after a 7-day exposure[11]. They reported 30% and 80% reductions in treated cells' tumor size imposed by TMZ and DOX, respectively.

Moreover, three other studies had both modeling and therapeutic approaches. For example, using hiPSC-derived neural progenitor cells with a dysregulated receptor tyrosine kinase and p53 signaling in vitro, Sancho-Martinez *et al.* modeled glioma tumor-initiating cells (GTIC). The authors represented that such dysregulated neural progenitor cells recapitulate features of GTIC in vitro. Besides, the authors of this study approved their results by observing highly aggressive tumors following in vivo transplantation of hiPSC-derived neural progenitor cells with a dysregulated receptor tyrosine kinase and p53 signaling. It is worthy of note that, at the final stage of the study, cultured GTICs derived from hiPSCs were treated with 101 anticancer compounds, and among all, three effective molecules were introduced for targeting GTICs in vitro[12]. In another investigation, Anastasaki *et al.*, in a modeling-therapeutic design, investigated engineered hiPSCs with NF1 loss and KIAA1549:BRAF fusion to provide a human LGG xenograft model in mice samples. They observed that neuroglial progenitors derived from engineered hiPSCs could potentially give rise to tumor cells maintaining LGG characteristics in vivo for a period of 6 months observation. Furthermore, in the second stage of the study, they found that MEK inhibitor (PD0325901) treatment could prevent hiPSC-derived LGG cells' survival and increase apoptosis in their both in vivo and in vitro settings[23]. Moreover, Susanto *et al.* generated iPSC-derived human neuroepithelial stem (NES) cells from a Gorlin syndrome patient carrying a germline mutation in the sonic hedgehog (SHH) receptor PTCH1[12,24]. The authors reported that Gorlin NES cells can form tumors representative of human medulloblastoma upon transplantation into the mouse cerebellum. In addition, they isolated and in vitro cultured tumor-isolated NES (tNES) cells from primary tumors and found that tNES cells can result in accelerated tumor formation and enhanced neurosphere formation. Furthermore, their results showed that Vismodegib treatment decreased the viability of both primary and secondary tNES cells in comparison to control NES cells suggesting the role of SHH pathway. Fig. 2 shows the summary of therapeutic compounds utilized in these studies.

iPSC for modeling brain tumors

Models using iPSC-derived organoids

Out of 22 studies, 16 administered iPSC technology for providing both in vitro and in vivo cellular models to better clarify brain tumor biology and pathology (Fig. 4). In this line, seven

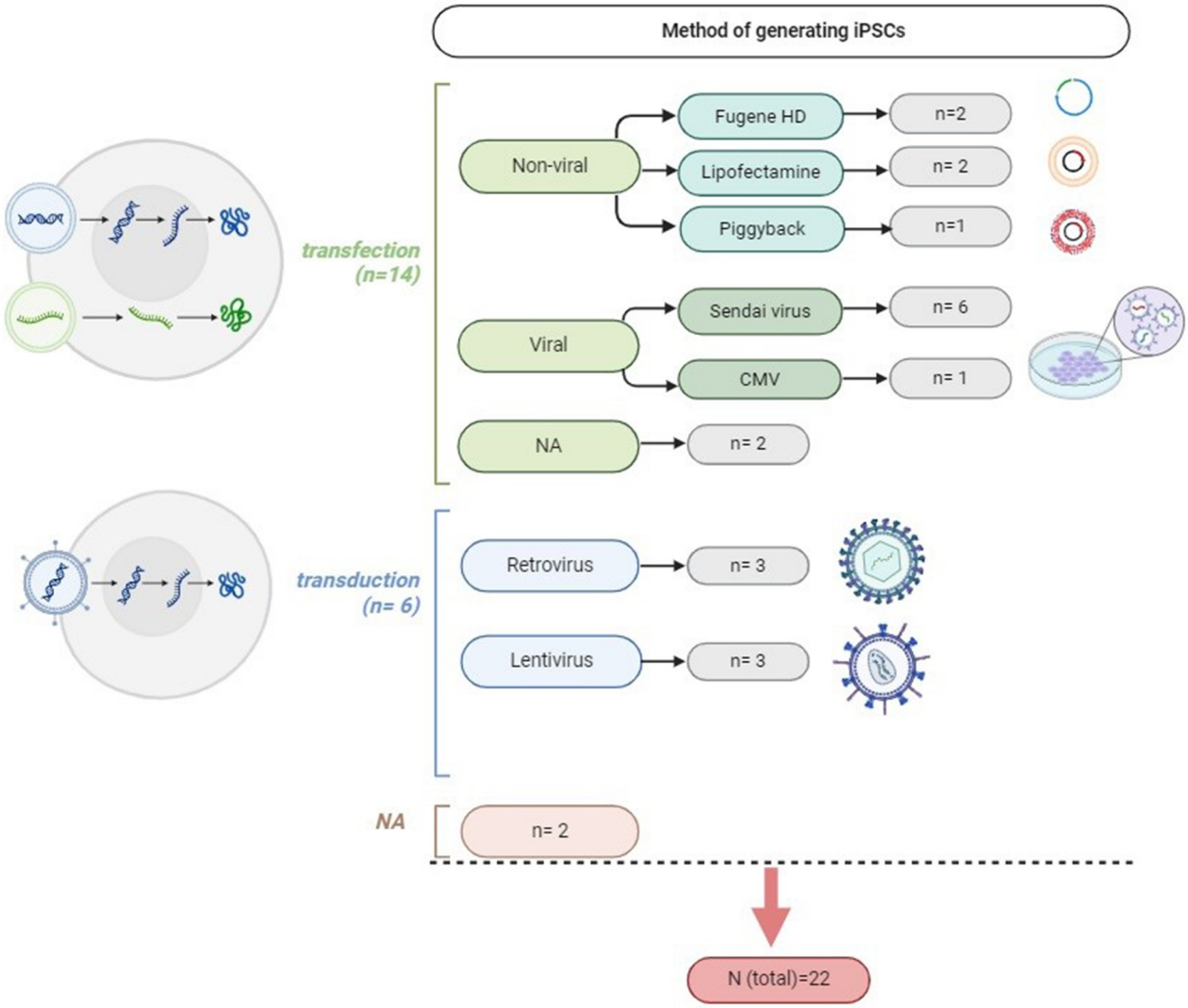

Figure 4. Classification and number of selected studies based on the main approach (modeling vs therapeutic) and design (in vivo vs in vitro). Illustrations used elements from BioRender.com (2021) (https://app.biorender.com/biorender-templates/, accessed on November 2023).

out of 16 studies administrated iPSC-derived human cerebral organoids for modeling brain tumor. It is worth mentioning that all seven organoid studies aimed to assess gliomablastoma. For example, Ogawa *et al*. utilized CRISPR/Cas9 technology to target an HRasG12V-IRES-tdTomato construct by homologous recombination into the TP53 locus and demonstrated the rapid invasion of transformed cells into surrounding iPSC-derived organoid structures[25]. In addition, the implanted organoid tumor cells into immune-deficient mice's brain exhibited an invasive phenotype. In another investigation, Bian *et al*. suggested a new 3D iPSC-derived in vitro model called a neoplastic cerebral organoid (neoCOR) and proved the capability of such model in demonstrating brain tumor biology. They utilized transposon and CRISPR-Cas9 to induce oncogenic mutations (by screening most relevant mutations detected in previous cancer genome projects) in cerebral iPSC-derived organoids and discover mutations leading to both central nervous system primitive neuroectodermal tumor-like and glioblastoma-like neoplasms[26]. Furthermore, Krieger *et al*. provided a scaffold for glioblastoma invasion by administrating iPSC-derived human cerebral organoids. They represented that human glioblastoma tumor cells implanted in cerebral organoids could recapitulate the in vivo characteristics of glioblastoma using tissue clearing technique and single-cell RNA sequencing of tumor cells before and after implantation within cerebral organoids[10]. In addition, Ballabio *et al*. identified Otx2 and c-MYC as the strong group medulloblastoma-3 inducers via an in vivo patient-specific screen and validated their findings by hiPSC-derived cerebellar organoids. In addition, the authors show that among all the putative onco-suppressors altered in Group 3 medulloblastoma, SMARCA4 significantly decreased Otx2/c-MYC tumorigenesis in ex vivo culture, as well as human cerebellar organoids[20].

In another study, Goranci-Buzhala developed an array of methods to provide rapid and efficient assaying of patient-derived glioma stem cell (GSC) invasion into human brain organoids generated from hiPSCs[27]. The authors claimed that such

assays were versatile to characterize different features of GSCs including invasion, integration, and interaction with mature neurons of brain organoids. Administering tissue clearing techniques and quantitative 3D imaging, they also reported that GSC invasion is inversely correlated with organoids' age. Also, they suggested that GI254023X, which is an inhibitor of ADAM10, could be an appropriate pharmacological agent to prevent invasion of GSCs into organoids. Moreover, Linkous *et al*. developed an ex vivo model system named cerebral organoid glioma model (GLICO) by administrating hESCs or hiPSCs-derived organoids to assess the invasion of patient-derived GSCs into host organoids[28]. The authors demonstrated that GSCs deeply invade and proliferate into the host tissue/organoid and form tumors prominently representing patient glioblastomas. Such rapid invasion is supported by an interconnected network of tumor microtubes according to their findings. Furthermore, Hwang *et al*. utilized patient-c-met-mutated h-iPSCs derived organoids to model glioblastoma (GBM) by the overexpression of c-met gene (one of the molecular features of glioblastoma)[29]. The authors showed neuronal- and GBM-related genes in c-met-mutated h-iPSCs after 90 days or more. Moreover, c-met-mutated h-iPSCs expressed high levels of glial fibrillary acidic protein and high levels of phospho-MET and phospho-STAT3, which are considered the typical markers of human glioblastomas. Also, authors suggested that TMZ has a preferential cytotoxicity toward c-met-mutated neuronal-like organoids and therefore can be an efficient therapeutic agent in glioblastoma models.

Models using non-organoid approaches

Other studies with a modeling approach used non-organoid iPSC-based models for investigating brain tumors. For example, Koga *et al*. administered the neural progenitor cells derived from genetically engineered human pluripotent stem cells (hiPSCs) and transfected them by Sendai virus to carry tumor-associated mutations proved by "The Cancer Genome Atlas project for glioblastoma" (GBM). The authors reported that such mutations could lead to high-grade gliomas with inter- and intra-tumor heterogeneities suggesting different glioblastoma molecular pathways[19]. In addition, Cancer *et al*. compared humanized models of SHH-subgroup medulloblastoma via MYCN overexpression in primary human embryonic hindbrain-derived neuroepithelial stem cells (hbNES) and iPSC-derived NES cells in terms of tumor formation, invasion, and dissemination[30]. The authors represented that iPSC-NES-derived medulloblastoma exhibited a more aggressive feature in comparison to hbNES-derived medulloblastoma upon xenografting. In addition, they discussed the roles of both Oct4 and mTOR signaling pathways in the aggressive phenotype of medulloblastoma models as the potential therapeutic targets in this population.

In another investigation, Xue *et al*. administered synthetic mRNA to generate hiPSC-derived neural precursors and transformed them with the MYC oncogene combined with p53 loss of function to generate MYC-driven medulloblastoma model. Also, the authors administered the marine compound Frondoside A (FA) as a cytotoxic agent to evaluate its antitumor effect on MYC-driven medulloblastoma-3. They found that FA positively suppresses MYC expression both in vitro and in vivo. Moreover, FA could improve antitumor function of microglia/macrophages and cytotoxic T lymphocytes in vivo[31]. Moreover, Terada *et al*. administered SMARCB1-deficient hiPSC-derived neural progenitor cells to model atypical teratoid/rhabdoid tumors. They observed that SMARCB1-deficient hiPSCs could confer a rhabdoid histology after transplantation into the mouse brain. Besides, they represented the activation of ESC-like gene and ESC-like DNA methylation in SMARCB1-deficient neural progenitor cells-derived tumors, which has been proved to be the cause of a poor prognosis in atypical teratoid/rhabdoid tumor[32].

In a different modeling study, Liu *et al*. investigated early genetic amplifications or deletions accompanying gliomagenesis in iPSCs predisposing to IDH1 mutation, but not containing such mutation[33]. The notion behind this study was the fact that cells carrying IDH1 mutation, which is considered as an early genetic alteration leading to LGG, are refractory to iPSC reprogramming induced by Yamanaka factors. In other words, by inducing Yamanaka factors, only the cells without IDH1 mutation (but before acquisition of such mutation) might go through the iPSC clones. So, analyzing the genetic substrate of such iPSC clones by array-based comparative genomic hybridization could shed more light on the early genetic alteration in LGGs. The authors reported the presence of regional chromosomal amplifications in the derived iPSC clones, which are also present in LGG cells.

In another investigation, Baliña *et al*. examined the possibility of urinary epithelial cells for generating iPSC lines from two children with LGG and metastatic brain tumor[34]. The authors demonstrated that iPSCs can be successfully generated from a small volume of urine cells of children with brain tumors, which is comparable to iPSCs derived from children without brain tumors in terms of quality and morphology. Moreover, such iPSC lines could efficiently have differentiated into functional mesenchymal stem/stromal cells with immunomodulatory properties, which provides an attractive approach for the treatment of children with brain tumors. Moreover, Ikemoto *et al*. generated hiPSCs from the fibroblasts of four patients with Gorlin syndrome (Gln-iPSCs) in order to assess the disease pathogens. The authors observed that in comparison to control iPSCs, Gln-iPSCs could manifest into medulloblastoma in 100% of teratomas developed from implantation into immunodeficient mice. They also showed loss of heterozygosity in the PTCH1 gene in one of the medulloblastomas, indicating the similarity between Gorlin syndrome and Gln-iPSCs[35]. In addition, Huang *et al*. investigated medulloblastoma formation in transduced human NES cells with MYCN in comparison to human Gorlin NES cells after orthotopic transplantation in mice[36]. They observed that the transcriptomes and patterns of DNA methylation from the former were generally more representative of human medulloblastoma in comparison to the latter. Moreover, Haag *et al*. assessed the effect of engineered iPSCs carrying an inducible H3.3-K27M allele on gliomagenesis of different neural cell types. They found that although such allele (which is upregulated in the vast majority of diffuse pontine glioma in children) would be fatal for iPSCs, it may lead to an increased proliferation of neural stem cells and also oligodendrocyte progenitor cells[18].

Risk of bias and quality assessment

The results of risk of bias assessment in all selected in vivo studies are presented in Table 2. Approximately one-third of

Table 2

Risk of bias in in vivo studies selected in the current systematic review based on SYRCLES' tool

		Selection bias			Performance bias		Detection bias		Attrition bias	Reporting bias	Other
	Study	1	2	3	4	5	6	7	8	9	10
1	Yamazoe *et al.*, 2014	No	No	No	?	?	No	?	?	?	Yes
2	Ignacio Sancho-Martinez *et al.*, 2016	?	?	?	?	?	?	?	?	?	No
3	Ogawa *et al.*, 2018	?	?	?	?	?	?	?	?	?	No
4	Huang *et al.*, 2019	No	?	?	Yes	?	?	?	?	?	No
5	Liu *et al.*, 2019	?	?	?	?	?	?	?	?	?	No
6	Terada *et al.*, 2019	?	Yes	?	Yes	?	Yes	?	?	?	Yes
7	Ikemoto *et al.*, 2020	No	?	?	?	?	?	?	?	?	No
8	Koga *et al.*, 2020	Yes	Yes	?	?	?	?	?	?	?	Yes
9	Tamura *et al.*, 2020	Yes	Yes	?	Yes	?	?	?	?	?	Yes
10	Haag *et al.*, 2021	Yes	Yes	?	Yes	?	?	?	?	?	Yes
11	Anastasaki *et al.*, 2022	Yes	Yes	Yes	Yes	Yes	?	?	?	?	Yes
12	Susanto *et al.*, 2019	No	?	?	Yes	?	?	?	?	?	No
13	Xue *et al.*, 2021	No	?	?	?	?	?	?	?	?	No
14	Ballabio *et al.*, 2020	?	?	?	?	?	?	?	?	?	No

SYRCLE, Systematic Review Center for Laboratory Animal Experimentation.

Yes = low risk of bias; No = high risk of bias; ? = Unclear bias. (1) Sequence generation, (2) Baseline characteristics, (3) Allocation concealment, (4) Random housing, (5) Blinding, (6) Random outcome assessment, (7) Blinding, (8) Incomplete outcome data, (9) selective outcome reporting, and (10) other sources of bias.

in vivo studies presented a low risk of bias considering selection and performance biases. However, regarding detection, attrition, and reporting biases, the risk was considered unclear for all in vivo studies due to the lack of sufficient information in all in vivo studies. Also, the detailed results of the in vitro studies are presented in Table 3. Selection bias, performance bias, and detection bias are assessed as probably low risk or definitely low risk in all in vitro studies. Also, similar to the in vivo studies, attrition bias was not reported in selected studies clearly.

Discussion

In the current study, we aimed to systematically review the data on the role of iPSC technology in human brain tumor

Table 3

Risk of bias in in vitro studies selected in the current systematic review based on Oral Health Assessment Tool

Study		Selection bias	Confounding bias	Performance bias	Detection bias	Attrition/exclusion bias	Selective reporting bias	Other bias
1	Yamazoe *et al.*, 2014	+	−	++	++	−	−	−
2	Ignacio Sancho-Martinez *et al.*, 2016	+	+	++	++	−	−	−
3	Bian *et al.*, 2018	+	+	++	++	−	−−	−
4	Ogawa *et al.*, 2018	++	+	++	++	−	−	−
5	Liu *et al.*, 2019	++	+	+	+	−	−	−
6	Plummer *et al.*, 2019	+	++	++	++	−	−	−
7	Terada *et al.*, 2019	+	++	++	++	−	−	−
8	Linkous *et al.*, 2019	+	++	++	++	−	−	−
9	Susanto *et al.*, 2019	+	++	++	++	−	−	−
10	Cancer *et al.*, 2019	+	++	++	+	−	−	−
11	Ikemoto *et al.*, 2020	+	+	++	++	−	−	−
12	Krieger *et al*, 2020	+	++	++	++	−	−	−
13	Goranci-Buzhala *et al.*, 2020	+	++	+	++	−	−	−
14	Hwang *et al.*, 2020	+	++	++	+	−	−	−
15	Ballabio *et al.*, 2020	+	+	++	++	−	−	−
16	Xue *et al.*, 2021	+	++	+	++	−	−	−
17	Anastasaki *et al.*, 2022	+	+	++	++	−	−	−
18	Baliña-Sánchez *et al.*, 2023	+	+	++	++	−	−	−

++ Definitely low risk of bias; + probably low risk of bias; − Probably high risk of bias or insufficient information; −− Definitely high risk of bias

pathogenesis, diagnosis, and therapeutic approaches. This novel technology has been utilized in various in vivo and in vitro studies since 2014; however, to our knowledge, it has not been systematically reviewed so far. We selected 22 studies based on our search strategy and inclusion and exclusion criteria. We then reviewed findings on the type of brain tumor, type of iPSC generation, source of primary cells, type of species within the brain tumor that has been studied (in vivo studies), and the main aim of the study (i.e., whether therapeutic or modeling approaches). Almost all of the selected studies (95%) administered iPSCs with a human source, except for one study that utilized mouse embryonic fibroblasts as their iPSC source. Different types of brain tumors have been investigated throughout the selected studies; however, glioblastoma and medulloblastoma have been the most frequent brain tumors, followed by glioma, diffuse intrinsic pontine glioma, LGG, and tretoid/rhabdoid tumors.

iPSC technology and brain tumor modeling

It is worth mentioning that the majority of selected studies (70%) have utilized iPSC technology to model different types of brain tumors either using organoid models, in-vivo, in-vitro design, or mixed in-vitro and in-vivo designs. The most common approach among modeling studies has been using non-organoid models (nine studies), followed by using iPSC-derived organoids (seven studies). Since the discovery of iPSCs, researchers and, in particular, neuroscientists have successfully utilized this technology to provide various types of brain disorders, either in 2D cultures to generate in vitro disease models or by transferring cells into a host brain in order to generate in vivo models[37].

In the current systematic review, we showed that more than half of the studies modeling a specific brain tumor using iPSC technology have used a non-organoid approach. Among them, there are studies that used engineered iPSCs to carry a gene for glioma or Gorlin syndrome[18,33,35]. The other studies utilized iPSCs to provide the nervous system's precursor cells such as neural progenitor cells or neuro-epithelial cells in order to induce different brain tumors such as medulloblastoma and glioblastoma, either in an in vitro or in vivo design[19,30,31,34,36]. Moreover, in the current review, we showed that less than half of modeling studies have administered iPSC-derived organoids. It is worth mentioning that all these studies assessed glioblastoma as the brain tumor of interest[10,25-29], except one study that investigated medulloblastoma[20]. The majority of them utilized iPSC-derived organoids as a host for brain tumor cells. CRISPR/Cas9 technology has been also administered in two studies in order to induce oncogenic mutations in organoid models[25,26].

In comparison to currently available models to study brain tumors such as GEMMs, PDXs, and even iPSC-derived 2D in vitro models, 3D organoid culture models are proving the most innovative possibilities for modeling human brain tumors[26]. Numerous advantages position iPSC-derived models as superior to other models in terms of human relevance, genetic diversity, and complexity, as well as ease of use. For instance, in contrast to GEMMs, iPSCs can be obtained from human cells, enabling the creation of models that carry the specific genetic and epigenetic alterations present in human tumors. Additionally, iPSC-derived models can be obtained from human cells with specific genetic backgrounds and then differentiated into various brain cell types, such as neurons and glial cells enabling the study of genetic variations and their impact on tumor biology. Moreover, regarding ease of use for high-throughput screening, iPSC-derived models can be expanded and differentiated in vitro, making them scalable. However, although PDXs, involve implanting human tumor tissues into immunocompromised mice, preserving the original tumor architecture and heterogeneity[38], these models require access to fresh human tumor tissues and involve significant resources and expertise, as they need to be propagated in immunocompromised mice. In the same vein, developing GEMMs can be time-consuming and expensive due to the need for breeding and maintaining genetically modified mice[39]. Moreover, although each model has its strengths and limitations, it seems that iPSC-derived models offer high human relevance and scalability, making them suitable for personalized medicine and high-throughput drug screening[40]. GEMMs provide valuable insights into genetic mechanisms but are limited by species differences. PDXs maintain human tumor characteristics but require significant resources and expertise to maintain. Given the above, models obtained via iPSCs may provide a versatile tool for providing unique insights into human-specific brain tumor pathology. Moreover, Bian *et al*. generated a new organoid-based model named neoCORs by recapitulating genetic deviations found in human brain tumors. They acclaimed that in comparison to previous models, neoCORs have the potential to represent many features of brain tumors such as cellular identities, transcriptomic profiles specific to cancer pathways, the ability to go through in vivo expansion/invasion[26], and in vivo structural organization. Besides, using such an engineered organoid model, the authors were able to model a primitive neuroectodermal tumor; a tumor for which no successful animal or in vitro model has existed so far[26].

Thus, although approximately half of the existing modeling studies on human brain tumors have so far used organoid-based models, it is of high importance for future studies working on brain tumor pathology or therapeutic approaches to take more advantage of iPSC-derived organoids. Besides, providing 3D models that allow the functional investigation of genetic deviations within the same genetic background is also of paramount importance to shed more light on cellular and molecular pathological pathways involved in brain tumors. Moreover, for reaching the next important step of preclinical investigations and drug screenings, in addition to basic studies of tumor pathology, it is essential for the model to contain both tumor and normal cells.

Nevertheless, in spite of the great opportunity that iPSC-derived brain organoids are providing for better understanding of brain disorders, particularly brain tumors, there are still some limitations that should be addressed and considered in future investigations. To name some, Khamis *et al*. have mentioned the cellular diversity of iPSCs, the inherent variability among different iPSC batches, and the lack of reproducibility as important remaining challenges in iPSC-derived organoid's application in disease modeling and drug screening in brain tumor research[9].

iPSC technology as a novel therapeutic approach for brain tumors

Our findings showed that a few studies administered iPSC technology either as a vehicle or a substrate to assess the effect of an interventional approach on a specified brain tumor. Approximately half of the articles in this category utilized iPSC as a vehicle for intervention[21,22]. For example, while in one of the studies, the killing effect of transduced iPSC-derived neural

stem cells on glioma has been assessed[21], in another study, the tumor tropic activity of iPSC-derived neural stem cells for glioma was assessed in both in vivo and in vitro designs[22]. Moreover, other studies with therapeutic aims in the current systematic review administered iPSCs as a substrate for intervention. In other words, IPSC-derived glioma models have been used in these studies to assess the antitumor effect of chemical compounds such as TMZ, DOX, MEK inhibitor, visamodegib, and other different anticancer compounds[11,12,23,24].

It is worth mentioning that in the current systematic review, all the studies with therapeutic aims have investigated glioma/glioblastoma as their brain tumor of interest. Although all these studies are preclinical investigations, their results are promising in showing the potential key role of iPSCs in novel therapeutic protocols for brain tumors in future. In particular, the transfer of antitumor genes by iPSCs can be a novel approach in the treatment of malignant gliomas[11,12,21-24]. Furthermore, researches are required to investigate the long-term function of iPSCs carrying suicide genes in vivo and the safety of transplanted iPSCs. Moreover, although gliomas have been the main focus of iPSC-based interventions in these studies, the proper grade and classification of different gliomas should also be considered in future studies. Such heterogeneity in gliomas is based not only on histological features but also on specific methylation profiles and genetic signaling pathways involved in gliomas (e.g., different genes such as IDH1, ATRX, TP53, CDK2A, BRAF, FGFR1, and PDGFRA)[2]. Gliomas, which account for more than 70% of all brain tumors[41], are brain tumors originating from glial cells and are divided into three major subgroups: astrocytoma, oligodendroglioma, and glioblastoma[42]. Despite the higher prevalence of gliomas in comparison to other brain tumors, there is also a need to investigate the therapeutic applications of iPSC technology in other brain tumors such as medulloblastoma in future studies.

Method of generating iPSCs

The method of generating iPSCs among the selected studies can be divided into two general categories: transduction and transfection. In the current systematic review, approximately one-third of selected studies used transduction, while the other two-thirds used transfection methods. While viral vectors can be used in both transduction (integrating) and transfection (integration-free) methods, non-viral tools are only administered in transfection (integration-free) methods. Among selected studies, lentivirus (50%) and retrovirus (50%) have been two viral vehicles utilized in the transduction studies. On the other hand, Sendai virus (88%) and cytomegalovirus (12%) have been viral vectors administered in the transfection articles. Furthermore, various non-viral reagents have also been utilized in the transfection studies including Fu gene HD (two studies)[10,18], lipofectamin (two studies)[12,19], and piggyback transposase (one study)[20].

Both transfection and transduction tools are common vehicles to deliver foreign genetic materials into the eukaryotic cells. Studies have consistently reported viral vectors as a highly effective method compared to non-viral transfection tools[43]. Regardless of the size of the genetic material or the target cell type, viral vectors are more invasive to the host cells and consequently are more efficient for integrating a specific nucleic acid sequence into a host cell. However, utilizing viral vectors is a noticeable concern in clinical and preclinical studies due to the unlimited mitosis potential resulted by the carried pluripotent genes to the host cell. Moreover, viral vectors may activate an immunogenic consequence in the cell[44]. On the other hand, non-viral vectors are less toxic and less immunogenic but result in a lower transfection efficiency of the primary somatic cells. Therefore, footprint-free iPSCs, which are produced by non-viral, non-integrating vectors, are novel, effective, and suggested vehicles for clinical and preclinical studies. This highlights the need for a shift from transduction methods to transfection, integration-free methods in the future studies of iPSC application in human disease therapies. For example, Borestrom et al. generated articular cartilage in vitro using footprint-free iPSCs, reducing safety concerns about genome insertion and deletion and making significant progress toward clinical application of iPSCs[45]. Interestingly, three out of seven selected studies with interventional approaches utilized transduction method to generate iPSCs. Lacking more safe integrating methods for generating iPSCs, future studies are highly recommended to produce and try footprint-free iPSCs for investigating brain tumor pathology and therapeutic strategies, which is a first step toward translational human studies.

Limitations

Despite the great inspiration that iPSC technology is bringing to the realm of brain tumors' pathology assessment and intervention, there are some limitations that should be addressed in future studies. For example, the differences in iPSC lines, protocols, primary cell sources, and experimental setups result in variations in neural precursor cells, as well as research outcomes that should be considered in future studies. Moreover, although in recent years, with further advances in 3D culture systems, iPSCs have been differentiated into 3D organoids including various types of brain cells and regions to mimic more complex features of brain in comparison to 2D in vitro models[37], most of the organoid systems are limited by the lack of vasculature system. Thus, in order to better represent specific characteristics of microvascular system involved in brain tumors, co-culture of organoid systems and endothelial cells might help future studies to overcome such limitations. Finally, it is worthy of note that future studies are supposed to consider the risk of different biases including baseline characteristics, performance, and reporting biases. Although almost all of the selected studies in the current systematic review have been published in high-quality journals, their general risk of bias assessment has failed to show powerful results.

Another limitation of the current study is the inclusion of a significant number of animal studies. While animal models are crucial for understanding disease mechanisms and testing therapeutic interventions, differences in genetic, molecular, and immunological responses between species often hinder the direct application of findings to human populations. This discrepancy can reduce the effectiveness and safety of treatments when transitioning from animal studies to human trials. Therefore, it is essential to address these limitations and highlight the need for developing more human-relevant models. Alternatives such as humanized mouse models, which incorporate human cells into animal hosts, or organoid models, which replicate human tissue architecture through 3D cultures, offer improved representation of human brain tumor biology. These advanced models provide a more accurate framework for studying human-specific tumor

behaviors and therapeutic responses, enhancing the translational potential of preclinical research in future studies.

Conclusion

To the best of our knowledge, this is the first systematic review on the role of iPSC technology in brain tumor studies. iPSCs as a novel technology could play a significant role in diagnosis and therapeutic methods for brain tumors. Due to the complexity of brain's anatomy and heterogeneity of brain tumors' genetics, the research in this area is complicated. However, iPSC-derived models could provide a more suitable in vitro and in vivo platforms to shed more light on brain tumors pathophysiology and therapeutic strategies. Specifically, as a novel 3D model, iPSC-derived organoids have been solving several important limitations in understanding brain tumors pathophysiology in comparison to 2D models. Nevertheless, there are still some limitations such as cellular diversity and inherent variability in iPSCs, which are capable to be improved in future studies. Also, iPSCs could be administered in therapeutic approaches for brain tumors, including deriving antitumor agents to target cells and testing different antitumor components. However, more research is also needed to evaluate the safety of iPSCs in in vivo studies, and the long-term behavior of engineered cells and their side effects on the host cells should be evaluated precisely as well.

Ethical approval

Ethics approval was not required for this systematic review.

Consent

Informed consent was not required for this systematic review.

Financial support and sponsorship

This study was not funded.

Author contribution

Conceptualization: M.S. and A.M. Methodology: M.S., T.T., P.J., A.A.H., and A.M. Formal analysis and investigation: M.S., P.J., and T.T. Writing—original draft preparation: M.S., P.J., and T.T. Writing—review and editing: M.S., T.T., P.J., A.A.H., M.F., and A.M. Supervision: A.M.

Conflicts of interest disclosure

The authors declare that they have no conflicts of interest related to this study.

Research registration unique identifying number (UIN)

This study did not involve any human participants and/or research studies.

Guarantor

Tina Taherkhani.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

[1] Aldape K, Brindle KM, Chesler L, *et al*. Challenges to curing primary brain tumours. Nat Rev Clin Oncol 2019;16(8):509–20.
[2] Antonica F, Aiello G, Soldano A, *et al*. Modeling brain tumors: a perspective overview of in vivo and organoid models. Front Mol Neurosci 2022;15:818696.
[3] Dobson THW, Gopalakrishnan V. Preclinical models of pediatric brain tumors-forging ahead. Bioengineering (Basel) 2018;5:81.
[4] Day CP, Merlino G, Van Dyke T. Preclinical mouse cancer models: a maze of opportunities and challenges. Cell 2015;163:39–53.
[5] Li Z, Langhans SA. In vivo and ex vivo pediatric brain tumor models: an overview. Front Oncol 2021;11:620831.
[6] Gould SE, Junttila MR, de Sauvage FJ. Translational value of mouse models in oncology drug development. Nat Med 2015;21:431–39.
[7] Luo J, Li P. Human pluripotent stem cell-derived brain organoids as in vitro models for studying neural disorders and cancer. Cell Biosci 2021;11(1):99.
[8] Rowe RG, Daley GQ. Induced pluripotent stem cells in disease modelling and drug discovery. Nat Rev Genet 2019;20(7):377–88.
[9] Khamis ZI, Sarker DB, Xue Y, *et al*. Modeling human brain tumors and the microenvironment using induced pluripotent stem cells. Cancers (Basel) 2023;15:1253.
[10] Krieger TG, Tirier SM, Park J, *et al*. Modeling glioblastoma invasion using human brain organoids and single-cell transcriptomics. Neuro Oncol 2020;22:1138–49.
[11] Plummer S, Wallace S, Ball G, *et al*. A human iPSC-derived 3D platform using primary brain cancer cells to study drug development and personalized medicine. Sci Rep 2019;9:1407.
[12] Sancho-Martinez I, Nivet E, Xia Y, *et al*. Establishment of human iPSC-based models for the study and targeting of glioma initiating cells. Nat Commun 2016;7:10743.
[13] Moher D, Liberati A, Tetzlaff J, *et al*. Preferred Reporting Items for Systematic reviews and Meta-Analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.
[14] Shea BJ, Reeves BC, Wells G, *et al*. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ 2017;358.
[15] Hooijmans CR, Rovers MM, de Vries RBM, *et al*. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol 2014;14:43.
[16] Wong WC, Cheung CS, Hart GJ. Development of a quality assessment tool for systematic reviews of observational studies (QATSO) of HIV prevalence in men having sex with men and associated risk behaviours. Emerg Themes Epidemiol 2008;5:1–4.
[17] Tran L, Tam DNH, Elshafay A, *et al*. Quality assessment tools used in systematic reviews of in vitro studies: a systematic review. BMC Med Res Methodol 2021;21:101.
[18] Haag D, Mack N, Benites Goncalves da Silva P, *et al*. H3.3-K27M drives neural stem cell-specific gliomagenesis in a human iPSC-derived model. Article. Cancer Cell 2021;39:407–422.e13.
[19] Koga T, Chaim IA, Benitez JA, *et al*. Longitudinal assessment of tumor development using cancer avatars derived from genetically engineered pluripotent stem cells. Nat Commun 2020;11:550.
[20] Ballabio C, Anderle M, Gianesello M, *et al*. Modeling medulloblastoma in vivo and with human cerebellar organoids. Nat Commun 2020;11:583.
[21] Tamura R, Miyoshi H, Morimoto Y, *et al*. Gene therapy using neural stem/progenitor cells derived from human induced pluripotent stem cells: visualization of migration and bystander killing effect. Hum Gene Ther 2020;31:352–66.
[22] Yamazoe T, Koizumi S, Yamasaki T, *et al*. Potent tumor tropism of induced pluripotent stem cells and induced pluripotent stem cell-derived

neural stem cells in the mouse intracerebral glioma model. Int J Oncol 2015;46:147–52.
[23] Anastasaki C, Chatterjee J, Cobb O, *et al*. Human induced pluripotent stem cell engineering establishes a humanized mouse platform for pediatric low-grade glioma modeling. Acta Neuropathol Commun 2022;10:120.
[24] Susanto E, Marin Navarro A, Zhou L, *et al*. Modeling SHH-driven medulloblastoma with patient iPS cell-derived neural stem cells. Proc National Acad Sci 2020;117:20127–38.
[25] Ogawa J, Pao GM, Shokhirev MN, *et al*. Glioblastoma model using human cerebral organoids. Cell Rep 2018;23:1220–29.
[26] Bian S, Repic M, Guo Z, *et al*. Genetically engineered cerebral organoids model brain tumor formation. Nat Methods 2018;15:631–39.
[27] Goranci-Buzhala G, Mariappan A, Gabriel E, *et al*. Rapid and efficient invasion assay of glioblastoma in human brain organoids. Cell Rep Jun 9 2020;31:107738.
[28] Linkous A, Balamatsias D, Snuderl M, *et al*. Modeling patient-derived glioblastoma with cerebral organoids. Cell Rep 2019;26: 3203–11.e5.
[29] Hwang JW, Loisel-Duwattez J, Desterke C, *et al*. A novel neuronal organoid model mimicking glioblastoma (GBM) features from induced pluripotent stem cells (iPSC). Biochim Biophys Acta Gen Subj 2020;1864:129540.
[30] Čančer M, Hutter S, Holmberg KO, *et al*. Humanized stem cell models of pediatric medulloblastoma reveal an Oct4/mTOR axis that promotes malignancy. Cell Stem Cell 2019;25:855–870.e11.
[31] Xue Y, Fu Y, Zhao F, *et al*. Frondoside A inhibits an MYC-driven medulloblastoma model derived from human-induced pluripotent stem cells. Mol Cancer Ther 2021;20:1199–209.
[32] Terada Y, Jo N, Arakawa Y, *et al*. Human pluripotent stem cell-derived tumor model uncovers the embryonic stem cell signature as a key driver in atypical teratoid/rhabdoid tumor. Cell Rep 2019;26:2608–2621.e6.
[33] Liu Z, Che P, Mercado JJ, *et al*. Characterization of iPSCs derived from low grade gliomas revealed early regional chromosomal amplifications during gliomagenesis. J Neurooncol 2019;141:289–301.
[34] Baliña-Sánchez C, Aguilera Y, Adán N., *et al*. Generation of mesenchymal stromal cells from urine-derived iPSCs of pediatric brain tumor patients. Front Immunol 2023;14:1022676.
[35] Ikemoto Y, Miyashita T, Nasu M, *et al*. Gorlin syndrome-induced pluripotent stem cells form medulloblastoma with loss of heterozygosity in PTCH1. Aging (Albany NY) 2020;12:9935–47.
[36] Huang M, Tailor J, Zhen Q, *et al*. Engineering genetic predisposition in human neuroepithelial stem cells recapitulates medulloblastoma tumorigenesis. Article. Cell Stem Cell 2019;25:433–446.e7.
[37] Logan S, Arzua T, Canfield SG, *et al*. Studying human neurological disorders using induced pluripotent stem cells: from 2D monolayer to 3D organoid and blood brain barrier models. Compr Physiol 2019;9:565–611.
[38] Abdolahi S, Ghazvinian Z, Muhammadnejad S, *et al*. Patient-derived xenograft (PDX) models, applications and challenges in cancer research. J Transl Med 2022;20:206.
[39] Das B, Evrard YA, Chen L, *et al*. Patient-derived organoid and cell culture models from the NCI patient-derived models repository (NCI PDMR) preserve genomic stability and heterogeneity of patient tumor specimens. Cancer Res 2020;80:3916–3916.
[40] Beghini DG, Kasai-Brunswick TH, Henriques-Pons A. Induced pluripotent stem cells in drug discovery and neurodegenerative disease modelling. Int J Mol Sci 2024;25:2392.
[41] Ohgaki H. Epidemiology of brain tumors. Methods Mol Biol 2009;472:323–42.
[42] Weller M, Wick W, Aldape K., *et al*. Glioma. Nat Rev Dis Primers 2015;1:15017.
[43] Hardee CL, Arévalo-Soliz LM, Hornstein BD, *et al*. Advances in non-viral DNA vectors for gene therapy. Genes (Basel) 2017;8:65.
[44] Gharaati-Far N, Tohidkia MR, Dehnad A, *et al*. Efficiency and cytotoxicity analysis of cationic lipids-mediated gene transfection into AGS gastric cancer cells. Artif Cells Nanomed Biotechnol 2018;46:1001–08.
[45] Boreström C, Simonsson S, Enochson L, *et al*. Footprint-free human induced pluripotent stem cells from articular cartilage with redifferentiation capacity: a first step toward a clinical-grade cell source. Stem Cells Transl Med 2014;3:433–47.