Cancer Pathogenesis and Therapy 2 (2024) 219–230

Contents lists available at ScienceDirect

Cancer Pathogenesis and Therapy

journal homepage: www.journals.elsevier.com/cancer-pathogenesis-and-therapy

Review article

Co-culture models for investigating cellular crosstalk in the glioma microenvironment

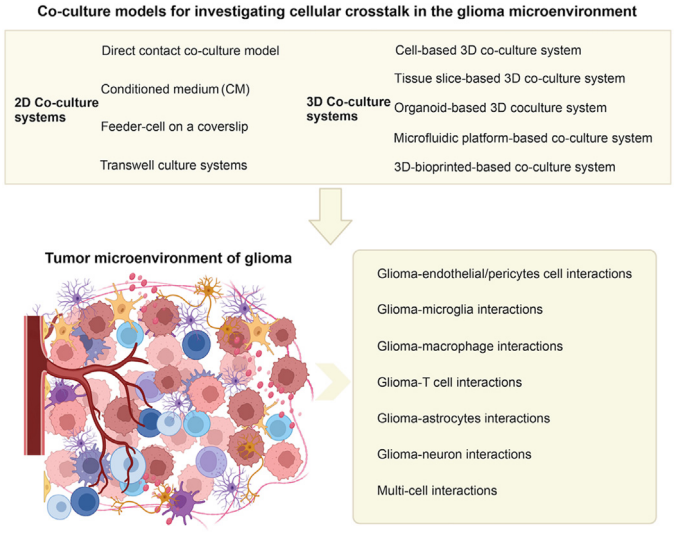
Xiaodong Niu [a], Yan Zhang [b,**], Yuan Wang [a,*]

[a] Department of Neurosurgery, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China
[b] National Clinical Research Center for Geriatrics, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China

HIGHLIGHTS

- The cellular composition of glioma encompasses a range of glioma cells alongside noncancerous cells.
- Common *in vitro* co-culture systems comprise two-dimensional (2D) and three-dimensional (3D) models.
- Within the glioma microenvironment, numerous intercellular interactions occur, giving rise to intricate networks.

GRAPHICAL ABSTRACT



ARTICLE INFO

Managing Editor: Peng Lyu

Keywords:
Glioma
Coculture techniques
Tumor microenvironment

ABSTRACT

Glioma is the most prevalent primary malignant tumor in the central nervous system (CNS). It represents a diverse group of brain malignancies characterized by the presence of various cancer cell types as well as an array of noncancerous cells, which together form the intricate glioma tumor microenvironment (TME). Understanding the interactions between glioma cells/glioma stem cells (GSCs) and these noncancerous cells is crucial for exploring the pathogenesis and development of glioma. To invesigate these interactions requires *in vitro* co-culture models that closely mirror the actual TME *in vivo*. In this review, we summarize the two- and three-dimensional *in vitro* co-culture model systems for glioma-TME interactions currently available. Furthermore, we explore common glioma-TME cell interactions based on these models, including interactions of glioma cells/GSCs with endothelial cells/

* Corresponding author: Department of Neurosurgery, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China.
** Corresponding author: National Clinical Research Center for Geriatrics, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China.
E-mail addresses: yanzhang@scu.edu.cn (Y. Zhang), wangyuan@scu.edu.cn (Y. Wang).

https://doi.org/10.1016/j.cpt.2023.11.002
Received 27 August 2023; Received in revised form 30 October 2023; Accepted 3 November 2023

pericytes, microglia/macrophages, T cells, astrocytes, neurons, or other multi-cellular interactions. Together, this review provides an update on the glioma-TME interactions, offering insights into glioma pathogenesis.

Introduction

Glioma is the most common type of primary malignant tumor of the central nervous system (CNS). Among its various forms, glioblastoma (GBM) is the most prevalent, representing more than half of all gliomas. Gliomas/GBM is characterized by rapid proliferation, aggressive invasiveness, and a high recurrence rate.[1,2] The prognosis deteriorates with increasing World Health Organization (WHO) glioma grade, with WHO grade 4 gliomas, such as GBM and diffuse midline glioma (DMG), exhibiting the poorest survival rates.[2]

Genomic and transcriptomic studies of bulk tumor tissues have revealed that GBMs can be categorized into three distinct subtypes: classical, proneural, and mesenchymal. Remarkably, these subtypes are associated with the accumulation of specific genetic mutations.[3–5] Single-cell transcriptomics has further deepened our understanding by unveiling that each tumor comprises a mixture of cells originating from various subtypes[3] and that the state of each cell corresponds to a distinct genetic alteration and epigenetic state, which significantly contributes to the diversification of tumor composition and characteristics.[6–10] Collectively, these findings indicate that glioma is a heterogeneous brain malignancy. It is characterized by a complex spectrum of genetic mutations, substantial inter- and intratumor variability, and a wide range of phenotypes.[3,11–13]

Glioma tissue has an intricate cellular makeup that comprises not only diverse cancer cell types but also a vast array of noncancerous cells. Together with the extracellular matrix (ECM), these cells form the distinctive glioma tumor microenvironment (TME) [Figure 1].[12–15] Besides glioma cells and glioma stem cells (GSCs), the glioma microenvironment encompasses endothelial cells (ECs), pericytes (PCs), microglia, macrophages, T cells, neurons, astrocytes, and other cells.[12,13,16,17] The intricate cellular interactions form a unique glioma tumor ecosystem. Most malignant tumors, including glioma, possess the ability to manipulate and restructure their microenvironment. Such modifications promote various tumorigenic traits, including the proliferation, invasion, migration, and treatment responsiveness of tumor cells. Concurrently, the TME undergoes dynamic shifts throughout glioma development and progression.[3,15,18,19] However, the current understanding of the microenvironmental factors influencing glioma is rudimentary, and the underlying mechanisms are not fully elucidated.

To fully elucidate the pathogenic mechanisms of glioma, multicellular/tissue culture systems are required. Although traditional *in vitro* culture systems using individual cell types, such as cancer cell lines or cancer stem cells (CSCs), can model the pathogenesis and progression of tumors to some degree, they cannot recapitulate the nuanced roles of TME cells in their natural, multifaceted environment *in vivo* and the complex interplay between glioma cells and other cells within the TME. Multicellular co-culture systems offer a more holistic approach and allow for a deeper exploration of the interactions between tumor cells and various non-tumor cells, shedding light on the pathological mechanisms of various cancers, including glioma.[20–23]

This review aims to provide a comprehensive summary of co-culture models, with a focus on two-dimensional (2D) and three-dimensional (3D) systems, and the diverse range of cellular interactions they facilitate. Further, we discuss common cell–cell interactions in the glioma TME, including those between glioma and immune cells, ECs, neurons, astrocytes, and stromal cells.

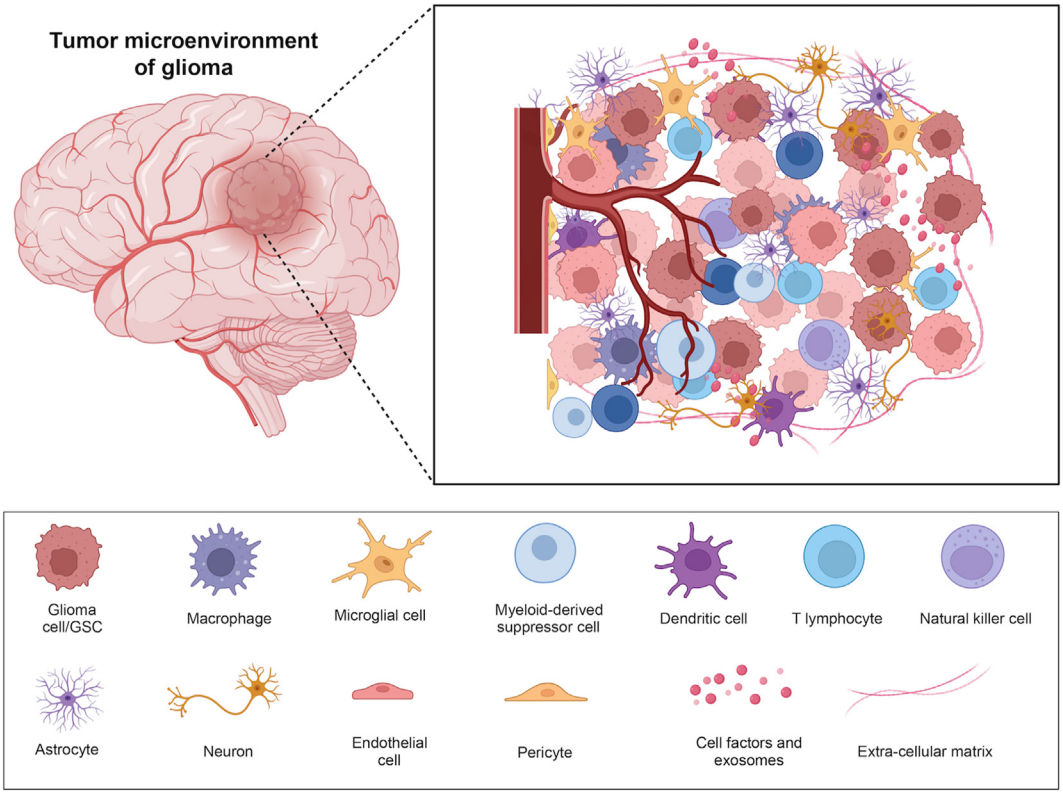


Figure 1. Schematic model of the glioma tumor microenvironment (TME). The cellular components of the glioma microenvironment include glioma cells and glioma stem cells (GSCs), endothelial cells, pericytes, microglia, macrophages, T cells, neurons, and astrocytes. The figure was created using BioRender (https://biorender.com/). GSC: Glioma stem cell; TME: Tumor microenvironment.

Co-culture systems for glioma

In vitro cell co-culture models can replicate the *in vivo* environment, facilitating accurate observation of cell–cell interactions and cell-microenvironment relations. They can provide in-depth insights into various cellular mechanisms, including cell proliferation, migration, differentiation, functionality, and vitality, as well as TME development and immunometabolic mechanisms.[21–23] *In vitro* glioma co-culture models can be largely categorized into 2D and 3D models based on model dimensionality and type [Figures 2 and 3].

Two-dimensional co-culture systems

In 2D culture models, various cell types can be cultured on a surface and often form a monolayer. To examine the impact of one cell type on another, two cell types can be grown together or separately (e.g., adhesion culture in culture dishes/flasks) in direct or indirect co-culture, respectively.[23] 2D co-culture systems can be further classified into direct and indirect contact co-culture models according to the contact ways of cell–cell interactions.

In the direct contact model, two or more cell types (e.g., GSCs or glioma cells) are mixed with another cell type (e.g., primary neurons or astrocytes) at a specified ratio or sequentially inoculated onto the same surface, while ensuring that the cells largely maintain their original form and function under the given co-culture conditions. This model has provided remarkable insights into neuronal-glioma cell interactions.[23]

Cell–cell interactions can also be indirectly mediated by secreted factors and extracellular vesicles (EVs) released into the microenvironment. To investigate such effects, a co-culture model that prevents direct contact between the different cell types is required. In indirect contact co-culture systems, physical contact between the cell types is avoided and cells interact via chemical cues in the culture medium. This type of system encompasses the use of conditioned medium (CM), concentrated EVs, or feeder cells on a coverslip, and Transwell culture systems [Figure 2].

Conditioned media contain secreted soluble cytokines and vesicles that occur in the glioma TME and exert autocrine and paracrine effects.[24–27] Cell culture supernatants can be used to elucidate the effects of secreted growth factors or exosomes on cell phenotypes. For example, CM collected from active neurons contained the soluble synaptic protein neuroligin-3 (NLGN3), which is an activity-regulated mitogen, and promoted the proliferation of high-grade glioma (HGG) by inducing the phosphatidylinositol 3-kinase (PI3K)-mammalian target of rapamycin (mTOR) pathway and feedforward NLGN3 expression in glioma cells.[28] Cells that secrete certain factors can also be plated on a coverslip to avoid direct contact with cells plated in a Petri dish.[29,30] This culture model is suitable for investigating the paracrine effects of cells under a specific culture condition. The two-layer Transwell co-culture culture system is widely used in experimental research on indirect intercellular interactions owing to its simplicity, standardization, and repeatability.[31–33] In the Transwell co-culture model, two types of cells are co-cultured on the bottom and top surfaces of the Transwell microporous membrane, respectively. For instance, co-culture of HPT cells (refers to mutant hNSCs with *PDGFRA D842V, H3K27M* overexpression, and *TP53* mutation) and neuronal stem cells (NSCs) in the Transwell system revealed that growth factors secreted by NSCs affect the cell behavior of HPT cells.[31]

Co-culture of different types of cells requires an appropriate culture medium that preserves the morphology and functionality of the cells. GSCs can alter their morphology when cultured under varying conditions. To maintain stemness in co-culture conditions, GSCs are typically cultured in serum-free neurobasal medium supplemented with stem cell growth factors, whereas primary ECs, astrocytes, and microglia are cultured in culture medium supplemented with fetal bovine serum (FBS).[23] The morphology of primary brain cells can also change in the GSC growth medium. Recently, Liu et al. investigated the optimal co-culture conditions for glioma cells and primary brain cells (neurons, astrocytes, microglia, and endothelial cells) *in vitro*.[23]

Three-dimensional co-culture systems

3D co-culture systems provide more reliable tools for studying complex cell interactions and pathological processes as they better

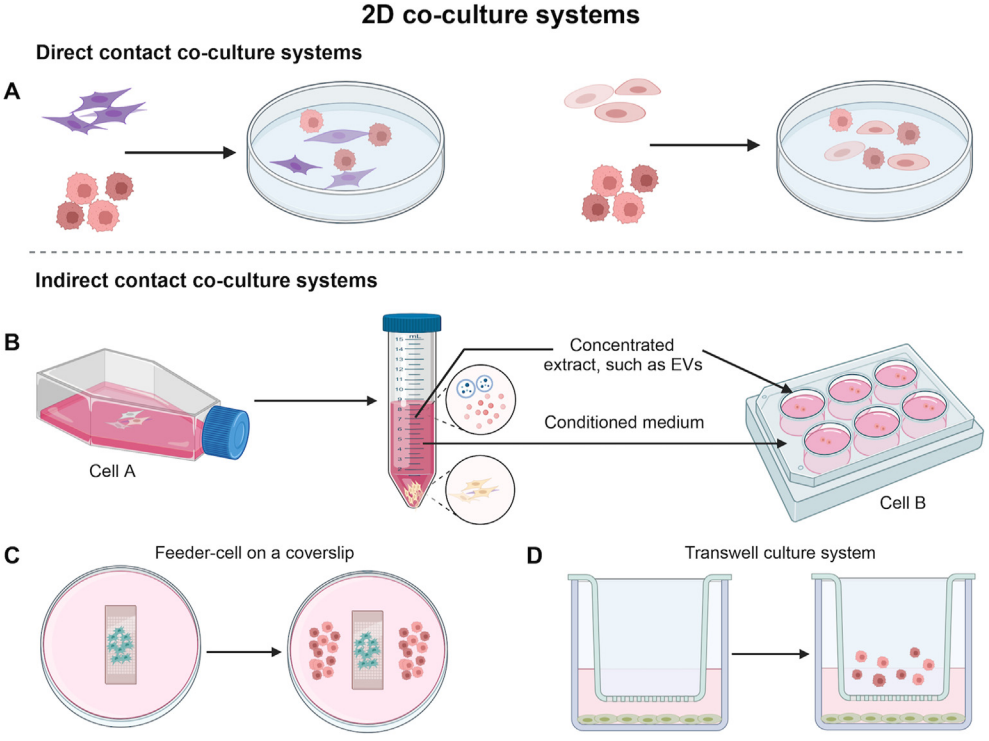


Figure 2. 2D co-culture systems for glioma. The 2D co-culture systems are categorized into direct contact co-culture (A) and indirect contact (B–D) co-culture models. The figure was created using BioRender (https://biorender.com/). 2D: Two-dimensional; EV: Extracellular vesicle.

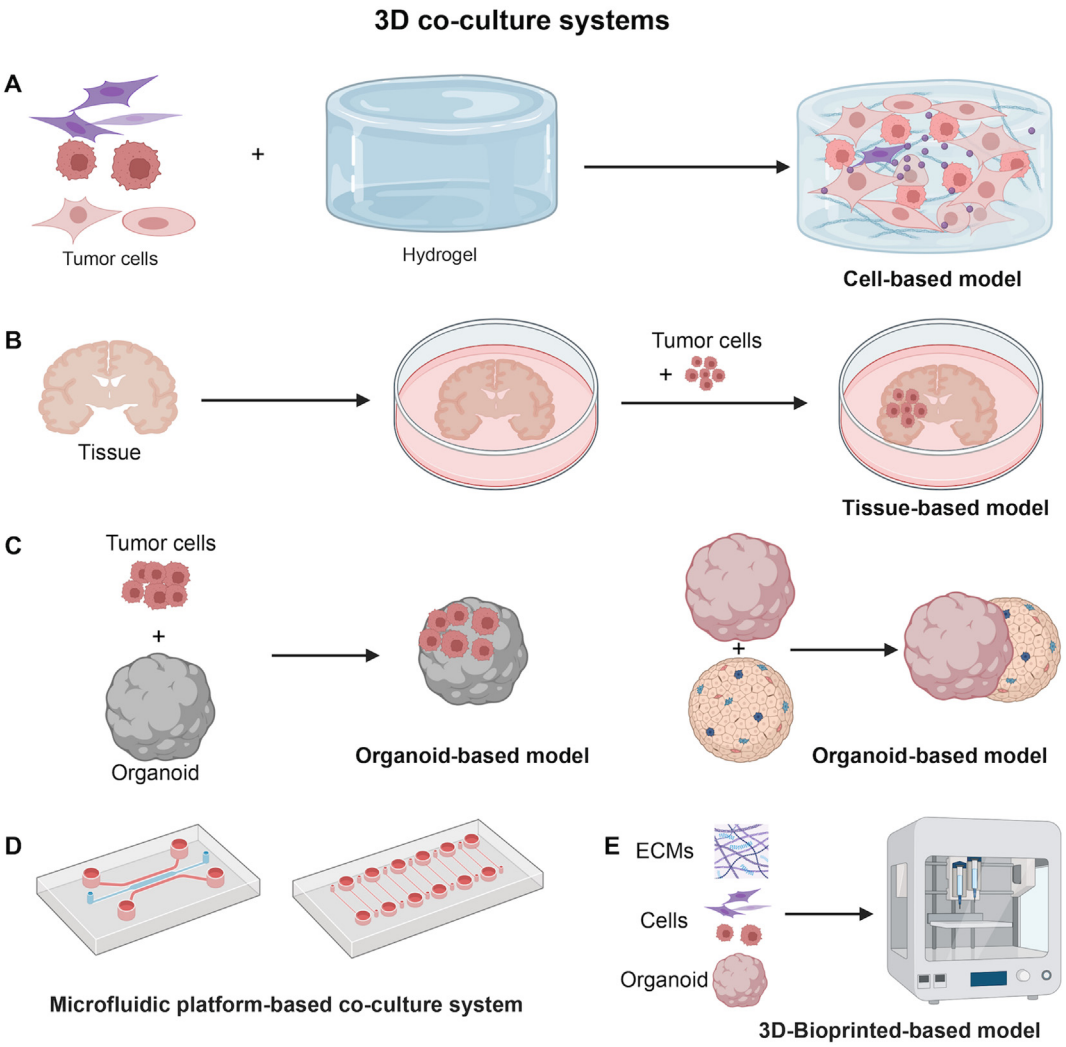


Figure 3. 3D co-culture systems for glioma. The 3D co-culture systems are classified into the following subtypes: cell-based (A); tissue slice-based (B); organoid-based (C); microfluidic platform-based (D); and 3D bioprinting-based (E). The figure was created using BioRender (https://biorender.com/). 3D: Three-dimensional; ECM: Extracellular matrix.

emulate *in vivo* environments than 2D systems. 3D co-culture models are used to explore cell activities, intercellular reactions, and cell functions, including cell proliferation, differentiation, invasiveness, and molecular characteristics.[23,34] Here, we classify the 3D co-culture model systems into the following subtypes: cell-based, tissue slice-based, organoid-based systems, microfluidic platform-based, and 3D bioprinting-based [Figure 3].

The cell-based 3D co-culture system is realized by encapsulating glioma cells, along with other cells, into biomaterial scaffolds such as hydrogels, which convert into a 3D structure post-gelation. The hydrogel guides cell behavior and facilitates material-cell interplay. Common hydrogel scaffolds encompass Matrigel, alginate, and decellularized tissue matrix (DTM).[23,35] Matrigel and alginate are natural hydrogels; Matrigel promotes cell growth and the onset of stem cell differentiation,[36–39] whereas alginate, which shares structural similarity with hyaluronic acid, a primary constituent of the brain ECM, is widely employed for 3D cell culture system construction.[40,41]

In 1991, Stoppini et al.[42] introduced brain slice culture as a new model for neuroscience research, which was further developed into current tissue slice-based 3D co-culture systems. This model is widely employed to examine various facets of neural development and tumor progression, including cell proliferation, migration, invasion, and apoptosis.[43,44] The model helps maintain morphological structure, tissue activity, and organ function akin to *in vivo* systems, providing a simulated *in vivo* microenvironment.[45,46]

3D cellular self-aggregates or organoids, which are typically derived from human pluripotent stem cells (hPSCs) or CSCs, are capable of maintaining multiple cellular lineages and preserving sophisticated cell–cell interactions.[47–49] The revolutionary organoid-based 3D models of the brain and brain tumor unleashed in 2013 and 2016, respectively,[50,51] are extensively employed in the study of a plethora of diseases, including glioma. 3D organoid models of GBM have been developed using various methods and are powerful models for studying glioma/GBM stem cell behavior and the effects of cell–cell and cell-microenvironment interactions on tumor growth and invasion.[47,48,52–54] Primary glioma cells, glioma cell lines, and GSCs can be co-cultured with different brain organoids, such as normal brain organoids and hPSC-induced TME cell organoids. Recently, Azzarelli et al. established a 3D GBM model by co-culturing GSCs with cerebral organoids that provided insights into cell fate identity as the tumor cells infiltrated the organoids.[55] Organoid-based co-culture model systems can be further developed to scrutinize cell–cell interactions and pathological processes prevalent in the TME.

Organ-on-a-chip- and microfluidic platform-based culture systems surmount the limitations of traditional *in vitro* and preclinical models.[56,57] Organ-on-a-chip platforms can more accurately predict the

efficacy of and reactions to drugs and therapies than *in vivo/in vitro* models.[57–61] These systems can be used to study cancer mechanisms, including tumor growth, migration, invasion, angiogenesis, intravasation and extravasation, and metastasis, as well as blood–brain barrier (BBB) permeability and drug responses.[56,62,63]

The innovative 3D bioprinting-based model system developed recently successfully emulates the complexity of human tumors and is revolutionizing preclinical cancer research.[64–70] This system, with unmatched control, precision, and reproducibility, can recreate intricate tumor-specific architectures according to TME characteristics through the use of live cells encapsulated in various biomaterials.[64,70] The system mirrors the actual pathological TME and emulates key *in vivo* cell functions, such as proliferation, invasiveness, and metastasis. The 3D bioprinted co-culture model has been successfully employed to study tumorigenesis mechanisms and drug responses in various diseases including, GBM.[67,69,70]

Although numerous studies have attempted to replicate the *in vivo* TME using 2D *in vitro* culture conditions, current conventional 2D models have limited usefulness and provide insufficient information because of their inherent limitations that affect cell phenotypes, metabolism, signaling, and treatment responses. Additionally, cells grown in 2D culture conditions exhibit distinct growth patterns, morphologies, and functions compared to those observed *in vivo*. 3D environments comprising cells organized in a specific spatial structure more closely resemble the actual TME *in vivo* than 2D systems and therefore enable a deeper understanding of the molecular biology of GBM and its treatment. The development of innovative *in vitro* 3D tumor models holds great potential for addressing current knowledge gaps and enhancing our understanding of GBM pathophysiology. In addition, these models can serve as screening tools to identify new candidate anti-GBM drugs. Table 1 summarizes the 2D and 3D *in vitro* co-culture systems used for studying the glioma TME.

Common glioma-tumor microenvironment cell interactions

Intercellular communication is essential for physiological homeostasis as well as in pathogenesis. In both *in vivo* and *in vitro* environments, cells communicate through both direct contact and via indirect mechanisms, including secretory factors and EVs. These mechanisms underline the complex biological and pathological networks, and cell interactions in the TME are emerging as a research focus.[23,27,71] However, *in vitro* models simulating these dynamics remain limited. An increasing number of studies investigate the interactions between tumor and non-tumor cells within the brain microenvironment and aim to simulate the glioma TME using co-culture systems.[23,72] In what follows, we summarize and discuss the common interactions between glioma and TME cells, including ECs, immune cells, astrocytes, and neurons, via direct and indirect pathways based on the diverse co-culture model systems [Table 2].

Glioma-endothelial cell/pericyte interactions

Gliomas, particularly GBMs, are highly vascularized neoplasms characterized by endothelial hyperplasia and microvascular proliferation. The endothelium/vascular tissues provide not only oxygen and nutrients but also a protective microenvironment to promote tumor growth.[27,72–74] Brain tumor vasculature is a composite of various cell types, including ECs, which are derived from endothelial progenitor cells and located in a perivascular or hypoxic niche, PCs, which originate from PC progenitor cells and surround the vasculature, and vascular smooth muscle cells.[12,27,75–77] Neovascularization within brain tumors leads to disorganized, convoluted, and leaky vascular networks that are modulated by various pro-angiogenic factors secreted by tumor cells, tumor-derived stromal cells (such as ECs and PCs), and inflammatory cells. Such angiogenic activity is often localized to the perivascular niche, where GSCs and ECs maintain proximal interactions within the glioma TME.[78]

ECs secrete vascular endothelial growth factor (VEGF) and other bioactive molecules that facilitate GSC infiltration and may induce the transdifferentiation of GSCs into ECs. Tumor-derived ECs maintain their endothelial identity while gaining mesenchymal characteristics, resulting in the promotion of angiogenesis, tumor proliferation and migration, and vascular permeability.[79–81] Additionally, ECs can promote tumor vasculature sprouting, relieve intra-tumoral hypoxia, and activate antitumor T cell immunity via phosphoglycerate dehydrogenase (PHGDH)-mediated endothelial metabolism.[82] EC-derived EVs drive proneural-to-mesenchymal reprogramming of GSCs, which involves the activation of matrix metalloproteinases (MMPs) and nuclear factor kappa

Table 1
Summary of 2D and 3D *in vitro* co-culture systems for glioma.

Co-culture systems		Advantages	Disadvantages	References
2D	Direct contact co-culture model	Homogeneity of cell populations Commercially available	Genotypic and phenotypic variations Different cell culture conditions considered Different cells not distinguished	23
	Conditioned medium	Easily acquired Simple operation	Different cells may be not adapted in a conditioned medium	24–27
	Transwell culture systems	Different cells can be distinguished Commercially available Simple operation Used in migration and invasiveness	The culture area and volume are small	31
3D	Cell-based 3D co-culture system	Homogeneity of cell populations Commercially available Suitable for high-throughput drug screening	Genotypic and phenotypic variations Very different growth conditions reported in the literature	23,35–39
	Tissue slice-based 3D co-culture system	Native tissue architecture Minimal experimental manipulation Enhanced physiological relevance	Limited tissue slice viability and lifespan Tissue slice variability may impact reproducibility	42–46
	Organoid-based 3D co-culture system	Mirrors the cell heterogeneity of the TME *in vivo* Suitable for studying the niche microenvironment Suitable for studying cancer cell invasion Cell populations can be genetically manipulated	Organoid composition may vary among experiments Results not easily reproducible	47,48,52–54
	Microfluidic platform-based co-culture system	Suitable for drug delivery and selectivity testing. BBB modeling	Technically challenging Accommodates a limited number of cell types	56,62,63
	3D bioprinting-based co-culture system	Possibility to build 3D microstructures of various cells Spatial organization and customizability	Technically challenging Lacks standardized protocols and validation criteria Costly	67,69,70

2D: Two-dimensional; 3D: Three-dimensional; BBB: Blood–brain barrier; TME: Tumor microenvironment.

Table 2
Common interactions between glioma and TME cells.

Cell interactions	Direction of interaction	Representative ways of interaction	References
Glioma-endothelial cell/pericyte interactions	Gliomas affect endothelial cells/pericytes	AnxA2-mediated binding of glioma-derived EVs to ECs fosters angiogenesis and promotes tumor progression	82,83
	Endothelial cells/pericytes affect gliomas	ECs can secrete VEGF and other bioactive molecules that facilitate GSC infiltration PC-derived CCL5 activates CCR5 on GBM cells, facilitating DNA damage repair after TMZ exposure	76,79–81
Glioma-microglia interactions	Gliomas affect microglia	Glioma cells harboring genetic alterations can interact with GAMs through the secretion of soluble mediators and EVs	95,100,105,106
	Microglia affect glioma	GAMs can regulate GSC self-renewal and stemness by secreting factors, including heparin-binding EGF-like growth factor, or via EVs	109–113
Glioma-macrophage interactions	Gliomas affect macrophages	Phagocytosis of glioma cells can transform BMDMs into M2-like macrophages and drive immunosuppression by expressing immune-checkpoint proteins (such as B7–H3 and PD-L1)	104
	Macrophages affect gliomas	TAMs can promote glioma cell proliferation and invasion through secreting factors or via EVs	109–114
Glioma-T cell interactions	Gliomas affect T cells	Phagocytosis of glioma cells by BMDMs can suppress the proliferation of activated T cells GBM-infiltrated myeloid cells express high levels of PD-L1 and inhibit T-cell function	104,123,133
	T cells affect gliomas	The exhaustion and dysfunction of T cells culminate into an immunosuppressive TME in glioma	122,131,132
Glioma-astrocyte interactions	Gliomas affect astrocytes	Glioma cells can deliver miRNAs to astrocytes via gap junctions for intracellular communication and increase glioma invasiveness	143
	Astrocytes affect gliomas	Tumor-associated astrocytes secrete IL-6, which activates the IL-6/p-STAT3 and Akt/p38/MAPK/ERK (1/2) pathways to create a suitable TME that promotes tumor progression	137,140–142
Glioma-neuron interactions	Gliomas affect neurons	Gliomas affect neuronal activity via various mechanisms, such as induced secretion of soluble factors, neurotransmitter release, TM formation, synaptogenesis promotion, and neuronal remodeling	26,153,154,158–160
	Neurons affect gliomas	Exosomes derived from cortical neurons bind selectively to gliomas and not glial cells, indicating indirect neuron-glioma communication	166–168

AnxA2: Annexin A2; BMDMs: Bone marrow-derived macrophages; B7–H3: B7 homolog 3 protein (also known as CD276); CCL5: C–C motif chemokine ligand 5; CCR5: C–C motif chemokine receptor 5; DNA: Deoxyribonucleic acid; ECs: Endothelial cells; EGF: Epidermal growth factor; ERK: Extracellular signal-regulated kinase; EVs: Extracellular vesicles; GAMs: Glioma-associated macrophages/microglia; GBM: Glioblastoma; GSCs: Glioma stem cells; IL-6: Interleukin-6; MAPK: Mitogen-activated protein kinase; PC: Pericyte; PD-L1: Programmed cell death protein ligand 1; PKB: protein kinase B (also known as Akt); p-STAT3: Phosphorylated signal transducer and activator of transcription 3; TAMs, Tumor-associated microglia; TM: Tumor microtube; TME: Tumor microenvironment; TMZ: Temozolomide; VEGF: Vascular endothelial growth factor.

B (NFκB) and the inactivation of NOTCH, concurrently altering chemotherapeutic sensitivity and promoting infiltrative growth.[27] Furthermore, glioma cells and GSCs release periostin (POSTN), which mediates angiogenesis via the activation of TANK-binding kinase 1 (TBK1) signaling in ECs, whereas Annexin A2 (AnxA2)-mediated binding of glioma-derived EVs to ECs fosters angiogenesis, thus promoting tumor progression.[82,83]

PCs are peri-endothelial vascular mural cells located on the abluminal wall, juxtaposed between the vascular feet of astrocytes and the endothelial basal membrane of microvessels. They delineate the perivascular layers that support the vasculature and play an integral role in structural and functional BBB integrity.[76,77,84–86] Emerging evidence posits PCs as a novel cell-mediated immunological defense in the brain. PCs express receptors for several types of inflammatory signals and secrete several chemokines and inflammatory mediators that regulate leukocyte recruitment and the inflammatory phenotype.[85,87] Zhang et al.[76] revealed that PC-derived C–C motif chemokine ligand 5 (CCL5) activates its receptor C–C motif chemokine receptor 5 (CCR5) on GBM cells, facilitating deoxyribonucleic acid (DNA) damage repair (DDR) after temozolomide exposure. Studies have suggested that injured or inflamed PCs can be transformed into activated mesenchymal stem cells (MSCs), which secrete molecules pivotal in immune modulation.[86,88] Dias et al.[75] unveiled that the genesis of fibrotic scarring by PCs is a conserved feature of various CNS lesions, including gliomas. Lucio et al.[89] revealed that anomalies in PC and EC proliferation, in conjunction with intussusceptive angiogenesis, contribute to the anomalous vessel architecture observed in GBM. Furthermore, GSCs are summoned toward ECs via the SDF-1/CXCR4 signaling axis and are then prompted to transdifferentiate into PCs by transforming growth factor-β (TGF-β), resulting in perivascular niche remodeling and tumor growth.[90]

Glioma-microglia interactions

Originating from peripheral myeloid cells, microglia are a critical component of innate immunity within the CNS. They are located in the brain parenchyma and function as tissue-resident macrophages.[91] They are a long-living cell population that maintains self-renewal capability and are ontogenically distinct from peripheral macrophages.[92–95] Microglia are indispensable for developmental and homeostatic brain functions and effective responses to infection, tissue damage, and neoplastic conditions.[96,97] Within the TME, glioma-associated macrophages/microglia are collectively termed glioma-associated microglia.[95,98] These microglia promote GBM growth and progression in direct and indirect manners.[95]

Upon infiltration into the glioma TME, macrophages and microglia undergo education and reprogramming by glioma/GBM cells. Specific mutations in tumor suppressor genes (TSGs), such as *PTEN*, *NF1*, and *TP53*,[99–101] regulate factors derived from GBM cells and the activation and amplification of oncogenes, including *EGFR* and the circadian locomotor output cycles kaput (*CLOCK*) gene.[99,102,103] Glioma cells harboring these unique genetic alterations can interact with microglia through the secretion of soluble factors and EVs. This interaction influences the characteristics (i.e., infiltration and polarization) of microglia, potentially facilitating GBM progression.[24,100,104]

PTEN is a TSG that antagonizes PI3K signaling in GBM and is mutated and/or deleted in 30–40 % of GBM cases.[99] PTEN deficiency in GBM cells may induce macrophage migration and infiltration. Mechanistically, PTEN deficiency in GBM cells activates PI3K/AKT signaling, which is essential for the production of soluble factors (e.g., LOX and WISP1) in GBM cells, and leads to the recruitment and maintenance of GAMs via the activation of macrophage β1 integrin signaling, resulting in GBM

progression.[95,100,105,106] Moreover, oncogenic *EGFR* amplification and activation, a notable characteristic of GBM, is observed in approximately 60 % of GBM cases.[102] Activated EGFR modulates the expression and function of various factors in GBM cells. For instance, EGFR can significantly increase hypoxia-enhanced carbonic anhydrase IX (CAIX) transcriptional activity and amplify tumor necrosis factor α (TNFα)-induced vascular cell adhesion molecule-1 (VCAM-1) expression in GBM cells/GSCs, potentiating macrophage adhesion, infiltration, and polarization.[102,107,108] Recent research indicates that microglia experience intense oxidative stress in the GBM tumor immune microenvironment (TIME), which induces nuclear receptor subfamily 4 group A member 2 (NR4A2)-dependent transcriptional activity in microglia and promotes tumor growth.[18]

Tumor-associated microglia can promote glioma cell proliferation and invasion, forming a positive feedback loop. In mouse and human GBM models, microglia promoted GSC self-renewal and stemness, resulting in tumor growth and therapy resistance, by secreting EVs or certain factors, including heparin-binding epidermal growth factor (EGF)-like growth factor, interleukin (IL)-12, IL-1β, CCL8, CCL5, VEGF, and IL-6.[109–113]

Glioma-macrophage interactions

During glioma pathogenesis, brain-infiltrating bone marrow-derived macrophages (BMDMs) originating from hematopoietic stem cells can infiltrate the brain parenchyma because the BBB is destroyed.[25,114,115] BMDMs are mainly located in perivascular and necrotic and ischemic tumor regions.[116] Although glioma-associated microglia and BMDMs differ in their origins and spatial distribution, they share immune-regulatory functions and express common markers, such as CD68, CD11b, and CX3C chemokine receptor 1 (CX3CR1).[25,117,118] In the glioma TME, these glioma-associated microglia and BMDMs are often collectively referred to as glioma-associated microglia/macrophages (GAMs), which constitute 30–50 % of the total cellular components.[95,98] Comprising both BMDMs and brain-resident microglia, GAMs exhibit heterogeneity in their subpopulations and phenotypes, ranging from pro-inflammatory to alternatively activated.[25,95,98] Increasing evidence suggests a complex interplay between GBM cells and GAMs, which influence each other in specific genetic backgrounds and the TME. These complex relationships encompass alterations in oncogene activation or TSG inactivation in GBM cells, which can modulate various facets of GAM biology, including migration, adhesion, and polarization. GAMs exert both direct and indirect effects to promote GBM growth and progression.[95]

In the glioma TME, glioma cells can alter BMDM phenotypes to promote tumor progression. A recent study revealed that phagocytosis of glioma cells by BMDMs generated double-positive TAMs, which were transformed into M2-like macrophages and drove immunosuppression by expressing immune-checkpoint proteins (such as CD276, programmed cell death protein ligand 1 [PD-L1], and programmed cell death protein ligand 2 [PD-L2]) and suppressing the proliferation of activated T cells.[104] However, another study demonstrated that soluble LRIG3 (sLRIG3) derived from glioma tumor cells can block M2 polarization of TAMs via interacting with NETO2, thus suppressing GBM progression.[24] In addition, TAMs can promote glioma cell proliferation and invasion by secreting certain factors or EVs.[109–114] CCL18 derived from GAMs promotes glioma cell growth and invasion through CCR8 and downstream acid phosphatase 5 (ACP5) signaling.[114] The effects of TAMs on glioma may involve various mechanisms, including the secretion of soluble factors, the release of exosomes, and direct cell–cell contact.[24,104,113,114,119–121]

Glioma-T cell interactions

T lymphocytes orchestrate cell-mediated antitumor immune responses by directly killing cancer cells and enhancing the antitumor capabilities of other immune cells.[122,123] T cells are classified into subtypes depending on their surface molecules and functions. These subtypes mainly include helper CD4+, regulatory CD4+, cytotoxic CD8+, natural killer T cells (NKT), and memory T cells.[123,124] In GBM patients, increased infiltration of T cells is associated with prolonged survival.[125] CD4+ T cells coordinate antigen-specific immunity through their high plasticity and cytokine-producing ability. Regulatory T cells (Tregs) are immunosuppressive T cells that can be recruited by CCL2/CCL22, and the cytokines IL-10 and TGF-β can inhibit the antitumor effect of T cells and promote GBM progression.[123,126] NKTs are a special subset of T cells that have T cell and NK cell receptors on their surface. They secrete large amounts of cytokines and chemokines, which play an important role in cancer immune regulation.[127,128] The antitumor immune actions of T cells include both effector functionality and the ability to infiltrate the TME.[122]

Within the glioma TME, T cells are often exhausted, posing a barrier to effective immunotherapy.[122] T cell exhaustion manifests as a progressive, sustained reduction in effector function, including alterations in cytokine production and proliferative capability, and may be driven by persistent antigen exposure and chronic T cell activation in cancers.[122] Increasing evidence suggests that T cells cannot function properly in GBM because they are inhibited by GBM cells, along with upregulated immunosuppressive checkpoints, extensive immunosuppressive cell infiltration, and impaired migration of T cells within the glioma TME.[122,123,129,130] The proliferative ability of exhausted T cells is largely modulated by inhibitory receptors such as programmed cell death protein-1 (PD-1), cytotoxic T lymphocyte-associated protein-4 (CTLA-4), and T cell immunoglobulin and mucin domain-3 (TIM-3).[122] Furthermore, TAMs, myeloid-derived suppressor cells (MDSCs), and dendritic cells infiltrating the glioma TME can directly or indirectly inhibit T cell function via secreted immunosuppressive factors, including TGF-β and IL-10.[122,131,132] Notably, infiltrating T cells in glioma typically express one or more immunosuppressive checkpoints, such as PD-1 and CTLA-4. GBM-infiltrated myeloid cells express high levels of PD-L1, resulting in the formation of the PD-L1/PD-1 complex, which further inhibits T-cell function.[123,133]

In addition, extrinsic factors in the glioma TME, including hypoxia, nutrient deprivation, T cell metabolic reprogramming and mitochondrial fitness, and metabolic by-products such as D-2-hydroxyglutarate (D-2HG), can drive T cell exhaustion.[130,134–136] In the glioma TME, cancer and noncancerous cells often adapt to and overcome these harsh conditions and therefore undergo alterations that result in transcriptomic, epigenetic, and metabolic impairment. Collectively, these factors drive T cell exhaustion and dysfunction, culminating in an immunosuppressive TME in glioma.

Glioma-astrocyte interactions

Astrocytes, which are complex glial cells abundantly present in the CNS, are highly abundant in the glioma TME and implicated in glioma pathogenesis.[137] In certain CNS pathologies, astrocytes transform into reactive counterparts characterized by hypertrophy and the upregulation of intermediate filaments composed of nestin, vimentin, and glial fibrillary acidic protein (GFAP) followed by the formation of contacts with other cells (e.g., glioma cells), a phenomenon known as "astrogliosis."[137–139] Reactive astrocytes propagate neuroinflammatory responses by secreting various agents, including cytokines, chemokines, ILs, nitric oxide (NO), and EVs.[137,140–142] The interplay between glioma cells and astrocytes is direct, via cell–cell junctions, or indirect, via paracrine signaling.[137,139,141,143]

Astrocytes take on a reactive phenotype with high GFAP expression when they are in contact with tumor cells. Evidence suggests that the induction of the reactive phenotype is mediated by the NF-κB signaling pathway. Upon treatment with recombinant RANKL (rRANKL) or lipopolysaccharide (LPS), which are well-known activators of NF-κB signaling, astrocytes displayed increased NF-κB activity because of the downregulation of IκBα and upregulation of GFAP, ultimately leading to

the reactive phenotype.[137,144] CCM from GBM cells inhibited the function of p53, a tumor suppressor that regulates the expression of proteins that are secreted to stimulate adjacent cells, in healthy $p53^{+/+}$ astrocytes.[145] The ECM of $p53^{+/-}$ astrocytes is richer in laminin and fibronectin than that of $p53^{+/+}$ astrocytes, which may aid the survival of GBM cells.[145,146] Glioma cells can deliver microribonucleic acids (miRNAs) to astrocytes via gap junctions to increase glioma invasiveness.[143] Reactive astrocytes upregulate channel protein connexin43 (Cx43), which is involved in gap junctions for direct cell communication.[147] In addition, Cx43 modulates Bcl-2 and Bax2 levels in glioma cells to inhibit the mitochondrial apoptotic response and block cytochrome C release from the mitochondria, preventing malignant cells from undergoing apoptosis.[148] Further, the Gln can be taken up by GBM cells, as observed in astrocyte/glioma co-culture.[149] Evidence suggests that tumor-associated astrocytes secrete IL-6, which activates the IL-6/phosphorylated signal transducer and activator of transcription 3 (p-STAT3) and Akt/p38/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) (1/2) pathways, thus increasing MMP2/MMP-14 expression, and trigger several oncogenic factors to create a suitable TME for tumor proliferation, invasion, and angiogenesis.[150,151] These findings were also made in an astrocyte/glioma co-culture system.[152] In conclusion, glioma cells and astrocytes interact to create a microenvironment that is suitable for glioma proliferation and invasion and protects them from the cytotoxic effects of antitumor drugs.

Glioma-neuron interactions

An increasing body of evidence suggests that glioma–neuron interactions are involved in the synaptic and functional integration of glioma into the brain network to promote tumor progression. In the glioma TME, there exists a dynamic reciprocity governing both glioma and neuronal function. This bidirectional interaction between glioma and neurons may be mediated by various mechanisms, including electrochemical synapses, secreted factors, tumor microtubes (TMs), and EVs.[26,28,153–158] Pioneering studies have indicated that gliomas affect neuronal activity via the secretion of soluble factors, neurotransmitter release, TM formation, synaptogenesis promotion, and neuronal remodeling within the TME.[26,153,154,158–160] Olfaction has been revealed to stimulate mitral and tufted (M/T) cells, which reciprocate sensory information from olfactory receptor neurons (ORNs), following activity-dependent insulin-like growth factor 1 (IGF-1) secretion. This in turn promotes gliomagenesis originating in oligodendrocyte precursor cells (OPCs), establishing a nexus between sensory experience and gliomagenesis via the sensory neuronal circuit, whereas the neuronal TME catalyzes the establishment of neuron-glioma synapses (NGSs), which are essential for bidirectional communication in the glioma infiltration zone.[155] Superimposed molecular and functional single-cell data recently revealed that neuronal mechanisms can govern GBM cell invasion and progression on multiple levels, implying that GBM dissemination and cellular heterogeneity are closely interlinked in glioma.[16] Neuronal activity implicated in the regulation of precursors, electrochemical signaling pathways, and neuronal secretion exerts a profound effect on glioma.[16,28,158,161,162]

Glioma/GSC cells can hijack glutamatergic and gamma-aminobutyric acid (GABA)ergic signaling in neurons to promote brain tumor invasion.[16,163,164] Peritumoral GABAergic interneurons display interictal-like activity, which is associated with pre-operative seizures in glioma patients and may result from high NKCC1 expression and low KCC2 expression. In co-cultured neurons/glioma, downregulated KCC2 expression in glioma elicited GABA-dependent depolarization in the neurons by increasing their intracellular Zn^{2+} concentration.[163] Remarkably, glioma-derived exosomes can alter the electrical properties of neuronal networks within the glioma TME.[154] In addition, gliomas can modulate surrounding neurons by expressing and releasing trophic factors. For instance, nerve growth factor (NGF) released by glioma cells has been shown to promote glutamatergic NGSs and thus promote glioma growth.[165] Neuronal activity-induced complex calcium signals in GBM cells and the activation of glutamatergic NGSs advance GBC invasiveness by stimulating the *de novo* formation and dynamics of TMs.[16,155] NLGN3 derived from neurons has proven necessary and sufficient for promoting robust HGG cell proliferation by inducing PI3K-mTOR pathway activity.[28,161]

EVs have an indispensable role in cell–cell interactions, including neuron-glioma interaction, in the brain TME.[166–168] Evidence suggests that exosomes derived from cortical neurons bind selectively to glioma and not glial cells, indicating indirect neuron-glioma communication via neuron-derived EVs (NEVs).[168] Exosome secretion is activity-dependent and specifically mediated by glutamatergic activity involving α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) and *N*-methyl-D-aspartate receptors (NMDARs).[166,167] In addition to NEVs, glioma can modulate neuronal functions via glioma-derived EVs (GEVs).[26] For instance, GEVs have been shown to increase the frequency of spontaneous synaptic responses in neuronal cells, thus increasing NLGN3 levels, which may facilitate glioma progression.[169]

Multi-cell interactions

The TME of various cancers, including glioma, is a labyrinth of multicellular interactions, forming a convoluted network.[170–172] For instance, CCL2 and colony-stimulating factor (CSF)1 from tumor-associated astrocytes dictate the recruitment of TAMs and foster a pro-tumorigenic macrophage phenotype. Furthermore, astrocyte-derived cholesterol has emerged as crucial for glioma cell survival. These findings illuminate the mechanistic roles of astrocytes in governing GBM pathogenicity by altering the immunological properties of the TME and indicate the non-oncogenic metabolic dependency of GBM on cholesterol.[170] Glycoprotein non-metastatic B (GPNMB), which is involved in regulating transcription factor regulons implicated in proneural-to-mesenchymal (PN-MES) subtype transition, is predominantly expressed on macrophages. Macrophages displaying elevated levels of this protein facilitate tumor progression via promoting PN-MES subtype transition and attenuate T cell activation through non-effective retention.[171]

Conclusions and perspectives

In vitro co-culture systems are often designed to simulate the intercellular interactions that occur *in vivo*. In the glioma TME, glioma cells/GSCs with different phenotypes and numerous different noncancerous cell types coexist and form a complex network. Investigating glioma-TME cell interactions using *in vitro* co-culture systems requires specific and complex culture conditions that meet the requirements of all cell types involved.[23] 2D and 3D cell co-culture systems are widely used, and new technologies, such as microfluidic devices and 3D bioprinting, which can more closely mimic the 3D microenvironment *in vivo*, are gradually being used for model construction. Notably, organoid-based and 3D models using novel technologies hold great potential for investigating cell–cell interactions, modeling various disease conditions, and facilitating drug screening and are powerful tools for controlling and analyzing cell interactions. This review aims to highlight the co-culture models based on *in vitro* 2D and 3D co-culture systems related to glioma cell-TME cell interactions, which has provided integrated insights into glioma pathogenesis and TME.

Current research primarily focuses on interactions between two cell types, which has provided insights into glioma pathogenesis. However, current *in vitro* co-culture models are limited in fully recapitulating glioma *in vivo*, mainly because of the high heterogeneity and intricacy of the TME. Future research should focus on fusing cell culture models with multifaceted techniques and devices to create more refined co-culture systems for cell interaction studies.

Funding

This work was supported by the National Natural Science Foundation of China, China (Nos. 82203456, 82173179, and 82273117), National

Key Research and Development Program of China, Stem Cell and Translational Research, China (No.2022YFA1105200), China Postdoctoral Science Foundation, China (No. 2022M712255), Science & Technology Department of Sichuan Province, China (Nos. 23ZYZYT S0150 and 2023NSFSC1868), and Postdoctoral Research Fund, West China Hospital, Sichuan University, China (No.2023HXBH070).

Authors contribution

Xiaodong Niu: conceptualization, analysis and interpretation, figure and tables preparation,writing-original draft & editing; Yan Zhang: conceptualization, writing-review & editing; Yuan Wang: conceptualization, writing-review & editing, supervision. All authors critically revised the paper and approved the final version.

Ethics statement

None.

Data availability statement

The datasets used in the current study are available from the corresponding author on reasonable request.

Conflict of 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.

Acknowledgments

None.

References

1. Ostrom QT, Price M, Neff C, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2016–2020. *Neuro Oncol*. 2023;25:iv1–iv99. https://doi.org/10.1093/neuonc/noad149.
2. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol*. 2021;23:1231–1251. https://doi.org/10.1093/neuonc/noab106.
3. Walentynowicz KA, Engelhardt D, Cristea S, et al. Single-cell heterogeneity of EGFR and CDK4 co-amplification is linked to immune infiltration in glioblastoma. *Cell Rep*. 2023;42:112235. https://doi.org/10.1016/j.celrep.2023.112235.
4. Verhaak RGW, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010;17:98–110. https://doi.org/10.1016/j.ccr.2009.12.020.
5. Wang Q, Hu B, Hu X, et al. Tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment. *Cancer Cell*. 2017;32:42–56. https://doi.org/10.1016/j.ccell.2017.06.003.
6. Neftel C, Laffy J, Filbin MG, et al. An integrative model of cellular states, plasticity, and genetics for glioblastoma. *Cell*. 2019;178:835–849. https://doi.org/10.1016/j.cell.2019.06.024.
7. Patel AP, Tirosh I, Trombetta JJ, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science*. 2014;344:1396–1401. https://doi.org/10.1126/science.1254257.
8. Feinberg AP, Levchenko A. Epigenetics as a mediator of plasticity in cancer. *Science*. 2023;379:eaaw3835. https://doi.org/10.1126/science.aaw3835.
9. Yang J, Xu J, Wang W, Zhang B, Yu X, Shi S. Epigenetic regulation in the tumor microenvironment: molecular mechanisms and therapeutic targets. *Signal Transduct Targeted Ther*. 2023;8:210. https://doi.org/10.1038/s41392-023-01480-x.
10. Niu X, Yang Y, Ren Y, Zhou S, Mao Q, Wang Y. Crosstalk between m6A regulators and mRNA during cancer progression. *Oncogene*. 2022;41:4407–4419. https://doi.org/10.1038/s41388-022-02441-4.
11. Lee JK, Wang J, Sa JK, et al. Spatiotemporal genomic architecture informs precision oncology in glioblastoma. *Nat Genet*. 2017;49:594–599. https://doi.org/10.1038/ng.3806.
12. Bikfalvi A, da Costa CA, Avril T, et al. Challenges in glioblastoma research: focus on the tumor microenvironment. *Trends Cancer*. 2023;9:9–27. https://doi.org/10.1016/j.trecan.2022.09.005.
13. Nicholson JG, Fine HA. Diffuse glioma heterogeneity and its therapeutic implications. *Cancer Discov*. 2021;11:575–590. https://doi.org/10.1158/2159-8290.CD-20-1474.
14. Ye Z, Ai X, Zhao L, Fei F, Wang P, Zhou S. Phenotypic plasticity of myeloid cells in glioblastoma development, progression, and therapeutics. *Oncogene*. 2021;40:6059–6070. https://doi.org/10.1038/s41388-021-02010-1.
15. Varn FS, Johnson KC, Martinek J, et al. Glioma progression is shaped by genetic evolution and microenvironment interactions. *Cell*. 2022;185:2184–2199. https://doi.org/10.1016/j.cell.2022.04.038.
16. Venkataramani V, Yang Y, Schubert MC, et al. Glioblastoma hijacks neuronal mechanisms for brain invasion. *Cell*. 2022;185:2899–2917. https://doi.org/10.1016/j.cell.2022.06.054.
17. Jain S, Rick JW, Joshi RS, et al. Single-cell RNA sequencing and spatial transcriptomics reveal cancer-associated fibroblasts in glioblastoma with protumoral effects. *J Clin Invest*. 2023;133:e147087. https://doi.org/10.1172/JCI147087.
18. Ye Z, Ai X, Yang K, et al. Targeting microglial metabolic rewiring synergizes with immune-checkpoint blockade therapy for glioblastoma. *Cancer Discov*. 2023;13:974–1001. https://doi.org/10.1158/2159-8290.CD-22-0455.
19. Hoogstrate Y, Draaisma K, Ghisai SA, et al. Transcriptome analysis reveals tumor microenvironment changes in glioblastoma. *Cancer Cell*. 2023;41:678–692. https://doi.org/10.1016/j.ccell.2023.02.019.
20. Duan J, Wang Y. Modeling nervous system tumors with human stem cells and organoids. *Cell Regen*. 2023;12:4. https://doi.org/10.1186/s13619-022-00150-7.
21. Goshi N, Morgan RK, Lein PJ, Seker E. A primary neural cell culture model to study neuron, astrocyte, and microglia interactions in neuroinflammation. *J Neuroinflammation*. 2020;17:155. https://doi.org/10.1186/s12974-020-01819-z.
22. Arora D, Gupta P, Jaglan S, Roullier C, Grovel O, Bertrand S. Expanding the chemical diversity through microorganisms co-culture: current status and outlook. *Biotechnol Adv*. 2020;40:107521. https://doi.org/10.1016/j.biotechadv.2020.107521.
23. Liu R, Meng X, Yu XX, et al. From 2D to 3D co-culture systems: a review of co-culture models to study the neural cells interaction. *Int J Mol Sci*. 2022;23:13116. https://doi.org/10.3390/ijms232113116.
24. Li Y, Wang W, Hou X, et al. Glioma-derived LRIG3 interacts with NETO2 in tumor-associated macrophages to modulate microenvironment and suppress tumor growth. *Cell Death Dis*. 2023;14:28. https://doi.org/10.1038/s41419-023-05555-z.
25. Lin C, Wang N, Xu C. Glioma-associated microglia/macrophages (GAMs) in glioblastoma: immune function in the tumor microenvironment and implications for immunotherapy. *Front Immunol*. 2023;14:1123853. https://doi.org/10.3389/fimmu.2023.1123853.
26. Hua T, Shi H, Zhu M, et al. Glioma-neuronal interactions in tumor progression: mechanism, therapeutic strategies and perspectives. *Int J Oncol*. 2022;61:104. https://doi.org/10.3892/ijo.2022.5394.
27. Adnani L, Kassouf J, Meehan B, et al. Angiocrine extracellular vesicles impose mesenchymal reprogramming upon proneural glioma stem cells. *Nat Commun*. 2022;13:5494. https://doi.org/10.1038/s41467-022-33235-7.
28. Venkatesh HS, Johung TB, Caretti V, et al. Neuronal activity promotes glioma growth through Neuroligin-3 secretion. *Cell*. 2015;161:803–816. https://doi.org/10.1016/j.cell.2015.04.012.
29. Ioannou MS, Liu Z, Lippincott-Schwartz J. A neuron-glia co-culture system for studying intercellular lipid transport. *Curr Protoc Cell Biol*. 2019;84:e95. https://doi.org/10.1002/cpcb.95.
30. Rathinam ML, Watts LT, Narasimhan M, Riar AK, Mahimainathan L, Henderson GI. Astrocyte mediated protection of fetal cerebral cortical neurons from rotenone and paraquat. *Environ Toxicol Pharmacol*. 2012;33:353–360. https://doi.org/10.1016/j.etap.2011.12.027.
31. Ren Y, Huang Z, Zhou L, et al. Spatial transcriptomics reveals niche-specific enrichment and vulnerabilities of radial glial stem-like cells in malignant gliomas. *Nat Commun*. 2023;14:1028. https://doi.org/10.1038/s41467-023-36707-6.
32. Appelt-Menzel A, Cubukova A, Metzger M. Establishment of a human blood-brain barrier co-culture model mimicking the neurovascular unit using induced pluripotent stem cells. *Curr Protoc Stem Cell Biol*. 2018;47:e62. https://doi.org/10.1002/cpsc.62.
33. Hira VV, Breznik B, Van Noorden CJ, Lah T, Molenaar RJ. 2D and 3D *in vitro* assays to quantify the invasive behavior of glioblastoma stem cells in response to SDF-1α. *Biotechniques*. 2020;69:339–346. https://doi.org/10.2144/btn-2020-0046.
34. Joseph JV, Blaavand MS, Daubon T, Kruyt FA, Thomsen MK. Three-dimensional culture models to study glioblastoma—current trends and future perspectives. *Curr Opin Pharmacol*. 2021;61:91–97. https://doi.org/10.1016/j.coph.2021.08.019.
35. Ma J, Huang C. Composition and mechanism of three-dimensional hydrogel system in regulating stem cell fate. *Tissue Eng Part B*. 2020;26:498–518. https://doi.org/10.1089/ten.TEB.2020.0021.
36. Absalan F, Pasandi MS, Ghasemi Hamidabadi H, et al. Matrigel enhances differentiation of human adipose tissue-derived stem cells into dopaminergic neuron. *Neurosci Lett*. 2021;760:136070. https://doi.org/10.1016/j.neulet.2021.136070.
37. Cui Y, Lee P, Reardon JJ, et al. Evaluating glioblastoma tumour sphere growth and migration in interaction with astrocytes using 3D collagen-hyaluronic acid hydrogels. *J Mater Chem B*. 2023;11:5442–5459. https://doi.org/10.1039/D3TB00066D.
38. Ye L, Lv W, He W, et al. Reduced malignant glioblastoma recurrence post-resection through the anti-CD47 antibody and temozolomide co-embedded in-situ hydrogel system. *J Contr Release*. 2023;359:224–233. https://doi.org/10.1016/j.jconrel.2023.05.046.

39. Ye L, Ji H, Liu J, et al. Carbon nanotube–hydrogel composites facilitate neuronal differentiation while maintaining homeostasis of network activity. *Adv Mater*. 2021; 33:e2102981. https://doi.org/10.1002/adma.202102981.
40. Moxon SR, Corbett NJ, Fisher K, Potjewyd G, Domingos M, Hooper NM. Blended alginate/collagen hydrogels promote neurogenesis and neuronal maturation. *Mater Sci Eng C*. 2019;104:109904. https://doi.org/10.1016/j.msec.2019.109904.
41. Chaicharoenaudomrung N, Kunhorm P, Promjantuek W, Heebkaew N, Rujanapun N, Noisa P. Fabrication of 3D calcium-alginate scaffolds for human glioblastoma modeling and anticancer drug response evaluation. *J Cell Physiol*. 2019;234:20085–20097. https://doi.org/10.1002/jcp.28608.
42. Stoppini L, Buchs P-A, Muller D. A simple method for organotypic cultures of nervous tissue. *J Neurosci Methods*. 1991;37:173–182. https://doi.org/10.1016/0165-0270(91)90128-M.
43. Hirata E, Yukinaga H, Kamioka Y, et al. *In vivo* fluorescence resonance energy transfer imaging reveals differential activation of Rho-family GTPases in glioblastoma cell invasion. *J Cell Sci*. 2012;125:858–868. https://doi.org/10.1242/jcs.089995.
44. Heasman SJ, Ridley AJ. Mammalian Rho GTPases: new insights into their functions from *in vivo* studies. *Nat Rev Mol Cell Biol*. 2008;9:690–701. https://doi.org/10.1038/nrm2476.
45. Lassiter CM, Gal JS, Becker S, Hartman NW, Grabel L. Embryonic stem cell-derived neural progenitors transplanted to the hippocampus migrate on host vasculature. *Stem Cell Res*. 2016;16:579–588. https://doi.org/10.1016/j.scr.2016.02.043.
46. Humpel C. Organotypic brain slice cultures: a review. *Neuroscience*. 2015;305: 86–98. https://doi.org/10.1016/j.neuroscience.2015.07.086.
47. Wen J, Liu F, Cheng Q, et al. Applications of organoid technology to brain tumors. *CNS Neurosci Ther*. 2023;29:2725–2743. https://doi.org/10.1111/cns.14272.
48. Mariappan A, Goranci-Buzhala G, Ricci-Vitiani L, Pallini R, Gopalakrishnan J. Trends and challenges in modeling glioma using 3D human brain organoids. *Cell Death Differ*. 2021;28:15–23. https://doi.org/10.1038/s41418-020-00679-7.
49. Xu R, Zhou X, Wang S, Trinkle C. Tumor organoid models in precision medicine and investigating cancer-stromal interactions. *Pharmacol Ther*. 2021;218:107668. https://doi.org/10.1016/j.pharmthera.2020.107668.
50. Lancaster MA, Renner M, Martin CA, et al. Cerebral organoids model human brain development and microcephaly. *Nature*. 2013;501:373–379. https://doi.org/10.1038/nature12517.
51. Hubert CG, Rivera M, Spangler LC, et al. A three-dimensional organoid culture system derived from human glioblastomas recapitulates the hypoxic gradients and cancer stem cell heterogeneity of tumors found *in vivo*. *Cancer Res*. 2016;76: 2465–2477. https://doi.org/10.1158/0008-5472.CAN-15-2402.
52. Mayhew CN, Singhania R. A review of protocols for brain organoids and applications for disease modeling. *Star Protoc*. 2023;4:101860. https://doi.org/10.1016/j.xpro.2022.101860.
53. Lago C, Gianesello M, Santomaso L, et al. Medulloblastoma and high-grade glioma organoids for drug screening, lineage tracing, co-culture and *in vivo* assay. *Nat Protoc*. 2023;18:2143–2180. https://doi.org/10.1038/s41596-023-00839-2.
54. Abdullah KG, Bird CE, Buehler JD, et al. Establishment of patient-derived organoid models of lower-grade glioma. *Neuro Oncol*. 2022;24:612–623. https://doi.org/10.1093/neuonc/noab273.
55. Azzarelli R, Ori M, Philpott A, Simons BD. Three-dimensional model of glioblastoma by co-culturing tumor stem cells with human brain organoids. *Biol Open*. 2021;10:bio056416. https://doi.org/10.1242/bio.056416.
56. Alves AH, Nucci MP, Mamani JB, et al. The advances in glioblastoma on-a-chip for therapy approaches. *Cancers*. 2022;14:869. https://doi.org/10.3390/cancers14040869.
57. Hassell BA, Goyal G, Lee E, et al. Human organ chip models recapitulate orthotopic lung cancer growth, therapeutic responses, and tumor dormancy *in vitro*. *Cell Rep*. 2017;21:508–516. https://doi.org/10.1016/j.celrep.2017.09.043.
58. Shi Y, He X, Wang H, et al. Construction of a novel blood brain barrier-glioma microfluidic chip model: applications in the evaluation of permeability and anti-glioma activity of traditional Chinese medicine components. *Talanta*. 2023;253: 123971. https://doi.org/10.1016/j.talanta.2022.123971.
59. Lee JH, Kim SK, Khawar IA, Jeong SY, Chung S, Kuh HJ. Microfluidic co-culture of pancreatic tumor spheroids with stellate cells as a novel 3D model for investigation of stroma-mediated cell motility and drug resistance. *J Exp Clin Cancer Res*. 2018; 37:4. https://doi.org/10.1186/s13046-017-0654-6.
60. Lin L, He Z, Jie M, Lin JM, Zhang J. 3D microfluidic tumor models for biomimetic engineering of glioma niche and detection of cell morphology, migration and phenotype change. *Talanta*. 2021;234:122702. https://doi.org/10.1016/j.talanta.2021.122702.
61. Marino A, Battaglini M, Carmignani A, et al. Magnetic self-assembly of 3D multicellular microscaffolds: a biomimetic brain tumor-on-a-chip for drug delivery and selectivity testing. *APL Bioeng*. 2023;7:036103. https://doi.org/10.1063/5.0155037.
62. Straehla JP, Hajal C, Safford HC, et al. A predictive microfluidic model of human glioblastoma to assess trafficking of blood–brain barrier-penetrant nanoparticles. *Proc Natl Acad Sci USA*. 2022;119:e2118697119. https://doi.org/10.1073/pnas.2118697119.
63. Guo QR, Zhang LL, Liu JF, et al. Multifunctional microfluidic chip for cancer diagnosis and treatment. *Nanotheranostics*. 2021;5:73–89. https://doi.org/10.7150/ntno.49614.
64. Albritton JL, Miller JS. 3D bioprinting: improving *in vitro* models of metastasis with heterogeneous tumor microenvironments. *Dis Model Mech*. 2017;10:3–14. https://doi.org/10.1242/dmm.025049.
65. Langer EM, Allen-Petersen BL, King SM, et al. Modeling tumor phenotypes *in vitro* with three-dimensional bioprinting. *Cell Rep*. 2019;26:608–623.e6. https://doi.org/10.1016/j.celrep.2018.12.090.
66. Molander D, Sbirkov Y, Sarafian V. 3D bioprinting as an emerging standard for cancer modeling and drug testing. *Folia Med (Plovdiv)*. 2022;64:559–565. https://doi.org/10.3897/folmed.64.e73419.
67. Tang M, Xie Q, Gimple RC, et al. Three-dimensional bioprinted glioblastoma microenvironments model cellular dependencies and immune interactions. *Cell Res*. 2020;30:833–853. https://doi.org/10.1038/s41422-020-0338-1.
68. Ma X, Qu X, Zhu W, et al. Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting. *Proc Natl Acad Sci USA*. 2016;113: 2206–2211. https://doi.org/10.1073/pnas.1524510113.
69. Chirivì M, Bearzi C, Rosa P, et al. Biomimetic keratin-coated gold nanoparticles for photo-thermal therapy in a 3D bioprinted glioblastoma tumor model. *Int J Mol Sci*. 2022;23:9528. https://doi.org/10.3390/ijms23179528.
70. Tang M, Rich JN, Chen S. Biomaterials and 3D bioprinting strategies to model glioblastoma and the blood–brain barrier. *Adv Mater*. 2021;33:e2004776. https://doi.org/10.1002/adma.202004776.
71. Winkler F, Venkatesh HS, Amit M, et al. Cancer neuroscience: state of the field, emerging directions. *Cell*. 2023;186:1689–1707. https://doi.org/10.1016/j.cell.2023.02.002.
72. Wei Z, Kale S, El Fatimy R, Rabinovsky R, Krichevsky AM. Co-cultures of glioma stem cells and primary neurons, astrocytes, microglia, and endothelial cells for investigation of intercellular communication in the brain. *Front Neurosci*. 2019;13: 361. https://doi.org/10.3389/fnins.2019.00361.
73. Ramachandran M, Vaccaro A, van de Walle T, et al. Tailoring vascular phenotype through AAV therapy promotes anti-tumor immunity in glioma. *Cancer Cell*. 2023; 41:1134–1151. https://doi.org/10.1016/j.ccell.2023.04.010.
74. Pang L, Dunterman M, Xuan W, et al. Circadian regulator CLOCK promotes tumor angiogenesis in glioblastoma. *Cell Rep*. 2023;42:112127. https://doi.org/10.1016/j.celrep.2023.112127.
75. Dias DO, Kalkitsas J, Kelahmetoglu Y, et al. Pericyte-derived fibrotic scarring is conserved across diverse central nervous system lesions. *Nat Commun*. 2021;12: 5501. https://doi.org/10.1038/s41467-021-25585-5.
76. Zhang XN, Yang KD, Chen C, et al. Pericytes augment glioblastoma cell resistance to temozolomide through CCL5-CCR5 paracrine signaling. *Cell Res*. 2021;31: 1072–1087. https://doi.org/10.1038/s41422-021-00528-3.
77. Zhou W, Chen C, Shi Y, et al. Targeting glioma stem cell-derived pericytes disrupts the blood-tumor barrier and improves chemotherapeutic efficacy. *Cell Stem Cell*. 2017;21:591–603. https://doi.org/10.1016/j.stem.2017.10.002.
78. Calabrese C, Poppleton H, Kocak M, et al. A perivascular niche for brain tumor stem cells. *Cancer Cell*. 2007;11:69–82. https://doi.org/10.1016/j.ccr.2006.11.020.
79. Soda Y, Marumoto T, Friedmann-Morvinski D, et al. Transdifferentiation of glioblastoma cells into vascular endothelial cells. *Proc Natl Acad Sci USA*. 2011;108: 4274–4280. https://doi.org/10.1073/pnas.1016030108.
80. Das S, Marsden PA. Angiogenesis in glioblastoma. *N Engl J Med*. 2013;369: 1561–1563. https://doi.org/10.1056/NEJMcibr1309402.
81. Huang M, Liu T, Ma P, et al. c-Met–mediated endothelial plasticity drives aberrant vascularization and chemoresistance in glioblastoma. *J Clin Invest*. 2016;126: 1801–1814. https://doi.org/10.1172/JCI84876.
82. Zhang D, Li AM, Hu G, et al. PHGDH-mediated endothelial metabolism drives glioblastoma resistance to chimeric antigen receptor T cell immunotherapy. *Cell Metabol*. 2023;35:517–534. https://doi.org/10.1016/j.cmet.2023.01.010.
83. Song YX, Li X, Nie SD, et al. Extracellular vesicles released by glioma cells are decorated by Annexin A2 allowing for cellular uptake via heparan sulfate. *Cancer Gene Ther*. 2023;30:1156–1166. https://doi.org/10.1038/s41417-023-00627-w.
84. Mitrofanova L, Hazratov A, Galkovsky B, et al. Morphological and immunophenotypic characterization of perivascular interstitial cells in human glioma: telocytes, pericytes, and mixed immunophenotypes. *Oncotarget*. 2020;11: 322–346. https://doi.org/10.18632/oncotarget.27340.
85. Pombero A, Garcia-Lopez R, Martínez S. Pericyte–glioblastoma cell interaction: a key target to prevent glioblastoma progression. *Cells*. 2023;12:1324. https://doi.org/10.3390/cells12091324.
86. von Tell D, Armulik A, Betsholtz C. Pericytes and vascular stability. *Exp Cell Res*. 2006;312:623–629. https://doi.org/10.1016/j.yexcr.2005.10.019.
87. Huizer K, Zhu C, Chirifi I, et al. Periostin is expressed by pericytes and is crucial for angiogenesis in glioma. *J Neuropathol Exp Neurol*. 2020;79:863–872. https://doi.org/10.1093/jnen/nlaa067.
88. Caplan AI. MSCs: the sentinel and safe-guards of injury. *J Cell Physiol*. 2016;231: 1413–1416. https://doi.org/10.1002/jcp.25255.
89. Díaz-Flores L, Gutiérrez R, González-Gómez M, et al. Disproportion in pericyte/endothelial cell proliferation and mechanisms of intussusceptive angiogenesis participate in bizarre vessel formation in glioblastoma. *Cells*. 2021;10:2625. https://doi.org/10.3390/cells10102625.
90. Cheng L, Huang Z, Zhou W, et al. Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. *Cell*. 2013;153:139–152. https://doi.org/10.1016/j.cell.2013.02.021.
91. McLaurin J, Almazan G, Williams K, Antel JP. Immortalization and characterization of rat microglial cells. *Neuropathol Appl Neurobiol*. 1995;21:302–311. https://doi.org/10.1111/j.1365-2990.1995.tb01064.x.
92. Wang G, Zhong K, Wang Z, et al. Tumor-associated microglia and macrophages in glioblastoma: from basic insights to therapeutic opportunities. *Front Immunol*. 2022; 13:964898. https://doi.org/10.3389/fimmu.2022.964898.
93. Korin B, Ben-Shaanan TL, Schiller M, et al. High-dimensional, single-cell characterization of the brain's immune compartment. *Nat Neurosci*. 2017;20: 1300–1309. https://doi.org/10.1038/nn.4610.

94. Crotti A, Ransohoff RM. Microglial physiology and pathophysiology: insights from genome-wide transcriptional profiling. *Immunity*. 2016;44:505–515. https://doi.org/10.1016/j.immuni.2016.02.013.
95. Xuan W, Lesniak MS, James CD, Heimberger AB, Chen P. Context-dependent glioblastoma–macrophage/microglia symbiosis and associated mechanisms. *Trends Immunol*. 2021;42:280–292. https://doi.org/10.1016/j.it.2021.02.004.
96. Ginhoux F, Greter M, Leboeuf M, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science*. 2010;330:841–845. https://doi.org/10.1126/science.1194637.
97. Harry GJ. Microglia during development and aging. *Pharmacol Ther*. 2013;139:313–326. https://doi.org/10.1016/j.pharmthera.2013.04.013.
98. Chen Z, Feng X, Herting CJ, et al. Cellular and molecular identity of tumor-associated macrophages in glioblastoma. *Cancer Res*. 2017;77:2266–2278. https://doi.org/10.1158/0008-5472.CAN-16-2310.
99. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2008;455:1061–1068. https://doi.org/10.1038/nature07385.
100. Chen P, Zhao D, Li J, et al. Symbiotic macrophage-glioma cell interactions reveal synthetic lethality in PTEN-null glioma. *Cancer Cell*. 2019;35:868–884. https://doi.org/10.1016/j.ccell.2019.05.003.
101. Zheng H, Ying H, Yan H, et al. Pten and p53 converge on c-Myc to control differentiation, self-renewal, and transformation of normal and neoplastic stem cells in glioblastoma. *Cold Spring Harbor Symp Quant Biol*. 2008;73:427–437. https://doi.org/10.1101/sqb.2008.73.047.
102. Fan QW, Cheng CK, Gustafson WC, et al. EGFR phosphorylates tumor-derived EGFRvIII driving STAT3/5 and progression in glioblastoma. *Cancer Cell*. 2013;24:438–449. https://doi.org/10.1016/j.ccr.2013.09.004.
103. Chen P, Hsu WH, Chang A, et al. Circadian regulator CLOCK recruits immune-suppressive microglia into the GBM tumor microenvironment. *Cancer Discov*. 2020;10:371–381. https://doi.org/10.1158/2159-8290.CD-19-0400.
104. Wu M, Wu L, Wu W, et al. Phagocytosis of glioma cells enhances the immunosuppressive phenotype of bone marrow–derived macrophages. *Cancer Res*. 2023;83:771–785. https://doi.org/10.1158/0008-5472.CAN-22-1570.
105. Hu B, Wang Q, Wang YA, et al. Epigenetic activation of WNT5A drives glioblastoma stem cell differentiation and invasive growth. *Cell*. 2016;167:1281–1295. https://doi.org/10.1016/j.cell.2016.10.039.
106. Tao W, Chu C, Zhou W, et al. Dual Role of WISP1 in maintaining glioma stem cells and tumor-supportive macrophages in glioblastoma. *Nat Commun*. 2020;11:3015. https://doi.org/10.1038/s41467-020-16827-z.
107. Huang BR, Liu YS, Lai SW, et al. CAIX regulates GBM motility and TAM adhesion and polarization through EGFR/STAT3 under hypoxic conditions. *Int J Mol Sci*. 2020;21:5838. https://doi.org/10.3390/ijms21165838.
108. An Z, Knobbe-Thomsen CB, Wan X, Fan QW, Reifenberger G, Weiss WA. EGFR cooperates with EGFRvIII to recruit macrophages in glioblastoma. *Cancer Res*. 2018;78:6785–6794. https://doi.org/10.1158/0008-5472.CAN-17-3551.
109. Shi Y, Ping YF, Zhou W, et al. Tumour-associated macrophages secrete pleiotrophin to promote PTPRZ1 signalling in glioblastoma stem cells for tumour growth. *Nat Commun*. 2017;8:15080. https://doi.org/10.1038/ncomms15080.
110. Hide T, Komohara Y, Miyasato Y, et al. Oligodendrocyte progenitor cells and macrophages/microglia produce glioma stem cell niches at the tumor border. *EBioMedicine*. 2018;30:94–104. https://doi.org/10.1016/j.ebiom.2018.02.024.
111. Tabu K, Liu W, Kosaku A, et al. Glioma stem cell (GSC)-derived autoschizis-like products confer GSC niche properties involving M1-like tumor-associated macrophages. *Stem Cell*. 2020;38:921–935. https://doi.org/10.1002/stem.3193.
112. Zhang X, Chen L, Dang WQ, et al. CCL8 secreted by tumor-associated macrophages promotes invasion and stemness of glioblastoma cells via ERK1/2 signaling. *Lab Invest*. 2020;100:619–629. https://doi.org/10.1038/s41374-019-0345-3.
113. Guo X, Pan Y, Xiong M, et al. Midkine activation of CD8+ T cells establishes a neuron–immune–cancer axis responsible for low-grade glioma growth. *Nat Commun*. 2020;11:2177. https://doi.org/10.1038/s41467-020-15770-3.
114. Huang Y, Motta E, Nanvuma C, et al. Microglia/macrophage-derived human CCL18 promotes glioma progression via CCR8-ACP5 axis analyzed in humanized slice model. *Cell Rep*. 2022;39:110670. https://doi.org/10.1016/j.celrep.2022.110670.
115. Guldner IH, Wang Q, Yang L, et al. CNS-native myeloid cells drive immune suppression in the brain metastatic niche through Cxcl10. *Cell*. 2020;183:1234–1248.e25. https://doi.org/10.1016/j.cell.2020.09.064.
116. Bowman RL, Klemm F, Akkari L, et al. Macrophage ontogeny underlies differences in tumor-specific education in brain malignancies. *Cell Rep*. 2016;17:2445–2459. https://doi.org/10.1016/j.celrep.2016.10.052.
117. Roesch S, Rapp C, Dettling S, Herold-Mende C. When immune cells turn bad-tumor-associated microglia/macrophages in glioma. *Int J Mol Sci*. 2018;19:436. https://doi.org/10.3390/ijms19020436.
118. Bennett ML, Bennett FC, Liddelow SA, et al. New tools for studying microglia in the mouse and human CNS. *Proc Natl Acad Sci USA*. 2016;113:E1738–E1746. https://doi.org/10.1073/pnas.1525528113.
119. Liu Y, Li X, Zhang Y, et al. An miR-340-5p-macrophage feedback loop modulates the progression and tumor microenvironment of glioblastoma multiforme. *Oncogene*. 2019;38:7399–7415. https://doi.org/10.1038/s41388-019-0952-x.
120. Azambuja JH, Ludwig N, Yerneni SS, Braganhol E, Whiteside TL. Arginase-1+ exosomes from reprogrammed macrophages promote glioblastoma progression. *Int J Mol Sci*. 2020;21:3990. https://doi.org/10.3390/ijms21113990.
121. Jiang Y, Zhao J, Xu J, et al. Glioblastoma-associated microglia-derived exosomal circKIF18A promotes angiogenesis by targeting FOXC2. *Oncogene*. 2022;41:3461–3473. https://doi.org/10.1038/s41388-022-02360-4.
122. Watowich MB, Gilbert MR, Larion M. T cell exhaustion in malignant gliomas. *Trends Cancer*. 2023;9:270–292. https://doi.org/10.1016/j.trecan.2022.12.008.
123. Wang H, Zhou H, Xu J, et al. Different T-cell subsets in glioblastoma multiforme and targeted immunotherapy. *Cancer Lett*. 2021;496:134–143. https://doi.org/10.1016/j.canlet.2020.09.028.
124. Masopust D, Schenkel JM. The integration of T cell migration, differentiation and function. *Nat Rev Immunol*. 2013;13:309–320. https://doi.org/10.1038/nri3442.
125. Keskin DB, Anandappa AJ, Sun J, et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature*. 2019;565:234–239. https://doi.org/10.1038/s41586-018-0792-9.
126. Cho HR, Kumari N, Thi Vu H, Kim H, Park CK, Choi SH. Increased antiangiogenic effect by blocking CCL2-dependent macrophages in a rodent glioblastoma model: correlation study with dynamic susceptibility contrast perfusion MRI. *Sci Rep*. 2019;9:11085. https://doi.org/10.1038/s41598-019-47438-4.
127. Ma C, Han M, Heinrich B, et al. Gut microbiome–mediated bile acid metabolism regulates liver cancer via NKT cells. *Science*. 2018;360:eaan5931. https://doi.org/10.1126/science.aan5931.
128. Tian L, Xu B, Chen Y, et al. Specific targeting of glioblastoma with an oncolytic virus expressing a cetuximab-CCL5 fusion protein via innate and adaptive immunity. *Nat Can (Ott)*. 2022;3:1318–1335. https://doi.org/10.1038/s43018-022-00448-0.
129. Krämer C, Kilian M, Chih YC, et al. NLGN4X TCR transgenic T cells to treat gliomas. *Neuro Oncol*. 2023. https://doi.org/10.1093/neuonc/noad172. [Ahead of print].
130. van Hooren L, Handgraaf SM, Kloosterman DJ, et al. CD103+ regulatory T cells underlie resistance to radio-immunotherapy and impair CD8+ T cell activation in glioblastoma. *Nat Can (Ott)*. 2023;4:665–681. https://doi.org/10.1038/s43018-023-00547-6.
131. Friedrich M, Hahn M, Michel J, et al. Dysfunctional dendritic cells limit antigen-specific T cell response in glioma. *Neuro Oncol*. 2023;25:263–276. https://doi.org/10.1093/neuonc/noac138.
132. Dubinski D, Wölfer J, Hasselblatt M, et al. CD4+ T effector memory cell dysfunction is associated with the accumulation of granulocytic myeloid-derived suppressor cells in glioblastoma patients. *Neuro Oncol*. 2016;18:807–818. https://doi.org/10.1093/neuonc/nov280.
133. Wang X, Guo G, Guan H, Yu Y, Lu J, Yu J. Challenges and potential of PD-1/PD-L1 checkpoint blockade immunotherapy for glioblastoma. *J Exp Clin Cancer Res*. 2019;38:87. https://doi.org/10.1186/s13046-019-1085-3.
134. Notarangelo G, Spinelli JB, Perez EM, et al. Oncometabolite D-2HG alters T cell metabolism to impair CD8+ T cell function. *Science*. 2022;377:1519–1529. https://doi.org/10.1126/science.abj5104.
135. Kersten K, Hu KH, Combes AJ, et al. Spatiotemporal co-dependency between macrophages and exhausted CD8+ T cells in cancer. *Cancer Cell*. 2022;40:624–638. https://doi.org/10.1016/j.ccell.2022.05.004.
136. Lu J, Xu Z, Duan H, et al. Tumor-associated macrophage interleukin-β promotes glycerol-3-phosphate dehydrogenase activation, glycolysis and tumorigenesis in glioma cells. *Cancer Sci*. 2020;111:1979–1990. https://doi.org/10.1111/cas.14408.
137. Guan X, Hasan MN, Maniar S, Jia W, Sun D. Reactive astrocytes in glioblastoma multiforme. *Mol Neurobiol*. 2018;55:6927–6938. https://doi.org/10.1007/s12035-018-0880-8.
138. Boccazzi M, Ceruti S. Where do you come from and what are you going to become, reactive astrocyte? *Stem Cell Invest*. 2016;3:15. https://doi.org/10.21037/sci.2016.05.02.
139. Henrik Heiland D, Ravi VM, Behringer SP, et al. Tumor-associated reactive astrocytes aid the evolution of immunosuppressive environment in glioblastoma. *Nat Commun*. 2019;10:2541. https://doi.org/10.1038/s41467-019-10493-6.
140. Gagliano N, Costa F, Cossetti C, et al. Glioma-astrocyte interaction modifies the astrocyte phenotype in a co-culture experimental model. *Oncol Rep*. 2009;22:1349–1356. https://doi.org/10.3892/or_00000574.
141. Nieland L, Morsett LM, Broekman MLD, Breakefield XO, Abels ER. Extracellular vesicle-mediated bilateral communication between glioblastoma and astrocytes. *Trends Neurosci*. 2021;44:215–226. https://doi.org/10.1016/j.tins.2020.10.014.
142. Zhang H, Zhou Y, Cui B, Liu Z, Shen H. Novel insights into astrocyte-mediated signaling of proliferation, invasion and tumor immune microenvironment in glioblastoma. *Biomed Pharmacother*. 2020;126:110086. https://doi.org/10.1016/j.biopha.2020.110086.
143. Hong X, Sin WC, Harris AL, Naus CC. Gap junctions modulate glioma invasion by direct transfer of microRNA. *Oncotarget*. 2015;6:15566–15577. https://doi.org/10.18632/oncotarget.3904.
144. Kim JK, Jin X, Sohn YW, et al. Tumoral RANKL activates astrocytes that promote glioma cell invasion through cytokine signaling. *Cancer Lett*. 2014;353:194–200. https://doi.org/10.1016/j.canlet.2014.07.034.
145. Biasoli D, Sobrinho MF, da Fonseca ACC, et al. Glioblastoma cells inhibit astrocytic p53-expression favoring cancer malignancy. *Oncogenesis*. 2014;3:e123. https://doi.org/10.1038/oncsis.2014.36.
146. Rangel LP, Costa DC, Vieira TC, Silva JL. The aggregation of mutant p53 produces prion-like properties in cancer. *Prion*. 2014;8:75–84. https://doi.org/10.4161/pri.27776.
147. Theodoric N, Bechberger JF, Naus CC, Sin WC. Role of gap junction protein connexin43 in astrogliosis induced by brain injury. *PLoS One*. 2012;7:e47311. https://doi.org/10.1371/journal.pone.0047311.
148. Gielen PR, Aftab Q, Ma N, et al. Connexin43 confers temozolomide resistance in human glioma cells by modulating the mitochondrial apoptosis pathway. *Neuropharmacology*. 2013;75:539–548. https://doi.org/10.1016/j.neuropharm.2013.05.002.
149. Tardito S, Oudin A, Ahmed SU, et al. Glutamine synthetase activity fuels nucleotide biosynthesis and supports growth of glutamine-restricted glioblastoma. *Nat Cell Biol*. 2015;17:1556–1568. https://doi.org/10.1038/ncb3272.

150. Chen W, Xia T, Wang D, et al. Human astrocytes secrete IL-6 to promote glioma migration and invasion through upregulation of cytomembrane MMP14. *Oncotarget*. 2016;7:62425–62438. https://doi.org/10.18632/oncotarget.11515.
151. Bharti R, Dey G, Mandal M. Cancer development, chemoresistance, epithelial to mesenchymal transition and stem cells: a snapshot of IL-6 mediated involvement. *Cancer Lett*. 2016;375:51–61. https://doi.org/10.1016/j.canlet.2016.02.048.
152. Chekhonin IV, Chistiakov DA, Grinenko NF, Gurina OI. Glioma cell and astrocyte co-cultures as a model to study tumor–tissue interactions: a review of methods. *Cell Mol Neurobiol*. 2018;38:1179–1195. https://doi.org/10.1007/s10571-018-0588-3.
153. De Silva MI, Stringer BW, Bardy C. Neuronal and tumourigenic boundaries of glioblastoma plasticity. *Trends Cancer*. 2023;9:223–236. https://doi.org/10.1016/j.trecan.2022.10.010.
154. Spelat R, Jihua N, Sánchez Triviño CA, et al. The dual action of glioma-derived exosomes on neuronal activity: synchronization and disruption of synchrony. *Cell Death Dis*. 2022;13:705. https://doi.org/10.1038/s41419-022-05144-6.
155. Venkatesh HS, Morishita W, Geraghty AC, et al. Electrical and synaptic integration of glioma into neural circuits. *Nature*. 2019;573:539–545. https://doi.org/10.1038/s41586-019-1563-y.
156. Venkataramani V, Tanev DI, Strahle C, et al. Glutamatergic synaptic input to glioma cells drives brain tumour progression. *Nature*. 2019;573:532–538. https://doi.org/10.1038/s41586-019-1564-x.
157. Oellers P, Schallenberg M, Stupp T, et al. A coculture assay to visualize and monitor interactions between migrating glioma cells and nerve fibers. *Nat Protoc*. 2009;4:923–927. https://doi.org/10.1038/nprot.2009.62.
158. Huang-Hobbs E, Cheng YT, Ko Y, et al. Remote neuronal activity drives glioma progression through SEMA4F. *Nature*. 2023;619:844–850. https://doi.org/10.1038/s41586-023-06267-2.
159. Dong W, Fekete A, Chen X, et al. A designer peptide against the EAG2–Kvβ2 potassium channel targets the interaction of cancer cells and neurons to treat glioblastoma. *Nat Can (Ott)*. 2023;4:1418–1436. https://doi.org/10.1038/s43018-023-00626-8.
160. Curry RN, Aiba I, Meyer J, et al. Glioma epileptiform activity and progression are driven by IGSF3-mediated potassium dysregulation. *Neuron*. 2023;111:682–695. https://doi.org/10.1016/j.neuron.2023.01.013.
161. Wang Y, Liu YY, Chen MB, et al. Neuronal-driven glioma growth requires Gαi1 and Gαi3. *Theranostics*. 2021;11:8535–8549. https://doi.org/10.7150/thno.61452.
162. Lim-Fat MJ, Wen PY. Glioma progression through synaptic activity. *Nat Rev Neurol*. 2020;16:6–7. https://doi.org/10.1038/s41582-019-0290-1.
163. Al Awabdh S, Donneger F, Goutierre M, et al. Gephyrin interacts with the K-Cl cotransporter KCC2 to regulate its surface expression and function in cortical neurons. *J Neurosci*. 2022;42:166–182. https://doi.org/10.1523/JNEUROSCI.2926-20.2021.
164. Wang J, Liu W, Xu W, et al. Comprehensive analysis of the oncogenic, genomic alteration, and immunological landscape of cation-chloride cotransporters in pan-cancer. *Front Oncol*. 2022;12:819688. https://doi.org/10.3389/fonc.2022.819688.
165. Rosso P, Fico E, Mesentier-Louro LA, et al. NGF eye administration recovers the TrkB and glutamate/GABA marker deficit in the adult visual cortex following optic nerve crush. *Int J Mol Sci*. 2021;22:10014. https://doi.org/10.3390/ijms221810014.
166. Gu J, Jin T, Li Z, et al. Exosomes expressing neuronal autoantigens induced immune response in antibody-positive autoimmune encephalitis. *Mol Immunol*. 2021;131:164–170. https://doi.org/10.1016/j.molimm.2020.12.034.
167. Sharma P, Mesci P, Carromeu C, et al. Exosomes regulate neurogenesis and circuit assembly. *Proc Natl Acad Sci USA*. 2019;116:16086–16094. https://doi.org/10.1073/pnas.1902513116.
168. Chivet M, Javalet C, Laulagnier K, Blot B, Hemming FJ, Sadoul R. Exosomes secreted by cortical neurons upon glutamatergic synapse activation specifically interact with neurons. *J Extracell Vesicles*. 2014;3:24722. https://doi.org/10.3402/jev.v3.24722.
169. Gao X, Zhang Z, Mashimo T, et al. Gliomas interact with non-glioma brain cells via extracellular vesicles. *Cell Rep*. 2020;30:2489–2500. https://doi.org/10.1016/j.celrep.2020.01.089.
170. Perelroizen R, Philosof B, Budick-Harmelin N, et al. Astrocyte immunometabolic regulation of the tumour microenvironment drives glioblastoma pathogenicity. *Brain*. 2022;145:3288–3307. https://doi.org/10.1093/brain/awac222.
171. Xiong A, Zhang J, Chen Y, Zhang Y, Yang F. Integrated single-cell transcriptomic analyses reveal that GPNMB-high macrophages promote PN-MES transition and impede T cell activation in GBM. *EBioMedicine*. 2022;83:104239. https://doi.org/10.1016/j.ebiom.2022.104239.
172. Zhu C, Chrifi I, Mustafa D, et al. CECR1-mediated cross talk between macrophages and vascular mural cells promotes neovascularization in malignant glioma. *Oncogene*. 2017;36:5356–5368. https://doi.org/10.1038/onc.2017.145.