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Decoding glioblastoma's diversity: are neurons part of the game?

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Highlights:

- GBM development mirrors neurodevelopment but exploits it for tumor progression
- snRNA-seq allows analysis of GBM - neurons interactions
- GBM interact with neurons, amplifying pro-tumor signals, such as hyperexcitability

Abstract:

Glioblastoma multiforme (GBM, WHO Grade 4) is a highly aggressive primary brain tumor with limited treatment options and a poor prognosis. A key challenge in GBM therapy lies in its pronounced heterogeneity, both within individual tumors (intratumoral) and between patients (intertumoral). Historically, neurons have been underexplored in GBM research; however, recent studies reveal that GBM development is closely linked to neural and glial progenitors, often mimicking neurodevelopmental processes in a dysregulated manner. Beyond damaging neuronal tissue, GBM actively engages with neurons to promote pro-tumorigenic signaling, including neuronal hyperexcitability and seizures.

Single-cell RNA sequencing (scRNA-seq) has revolutionized our understanding of the tumor microenvironment (TME), uncovering the critical roles of immune cells, endothelial cells, and astrocytes in tumor progression. However, technical limitations of scRNA-seq hinder its ability to capture the transcriptomes of neurons, necessitating the use of single-nucleus RNA sequencing (snRNA-seq) to study these interactions at single-cell resolution. This work collects the emerging insights of glioblastoma-neuron interactions, focusing on how GBM exploits neurodevelopmental pathways and reshapes neuronal networks. Moreover, we perform bioinformatic analysis of publicly available snRNA-seq datasets to propose putative cell-cell interactions driving glioma-neuronal dynamics. This study delineates key signaling pathways and underscores the need for further investigation to evaluate their potential as therapeutic targets.

Keywords: glioblastoma, neurons, single-nucleus RNA sequencing, cell communication, neuron-glioma interactions, tumor heterogeneity, GSC, tumor microenvironment

1. Introduction

Historically, neurons were largely overlooked in glioblastoma multiforme (GBM) research, often viewed merely as collateral damage in tumor growth. However, recent discoveries have revealed that GBM development is closely linked to neural and glial progenitors, with some aspects of tumor progression mirroring normal neurodevelopment. While GBM may reflect certain neurodevelopmental cues, this resemblance is more like an image in a distorting mirror, as the mechanisms involved are often hijacked to promote tumor growth. In recent years, considerable attention has been drawn to the interactions between GBM and neurons. It is now recognized that a growing tumor not only destroys surrounding neuronal tissue but also actively communicates with it, triggering pro-tumorigenic signaling. Evidence has shown that glioma cells can induce neuronal hyperexcitability and seizures by secreting glutamate and synaptogenic factors [1,2]. Moreover, some antiepileptic drugs were shown to affect GBM survival [3]. This study focuses on the interactions between GBM cells and neurons, particularly in the context of how GBM hijacks neurodevelopmental pathways, emphasizing insights from single-cell resolution data. While GBM-immune cell interactions are well-documented, they will not be the primary focus here. Interestingly, neurons within GBM tissue samples were under-studied, mainly due to technical limitations in single-cell sequencing. Most single-cell studies rely on scRNA-seq, which struggles to capture the transcriptomes of neurons, making snRNA-seq a more suitable approach. Due to their complex morphology and size, neurons are often lost during the library preparation stage in many current scRNA-seq technologies. This study summarises the existing knowledge and bridges the gap in understanding the intricate interactions between GBM cells and neurons. To address some of the limitations in our current understanding, we propose a hybrid article format, combining a traditional review with a re-analysis of existing snRNA-seq datasets [4]. Here, we highlight potential GBM-neuron cell interactions that may have been missed due to the technical constraints of single-cell technologies.

2. Glioblastoma heterogeneity - from bulk to sc-RNAseq (from subtypes to cellular heterogeneity)

Glioblastoma multiforme (GBM, WHO Grade 4) is a highly aggressive primary brain tumor with a poor prognosis [5,6]. One of the main challenges in GBM treatment is its remarkable heterogeneity, both within individual tumors (intratumoral) and across patients (intertumoral) [7,8]. Moreover, treatment of recurrent GBM remains highly challenging, with no standardized second-line therapies that could significantly improve patients' survival. Many therapies effective in other cancers, such as targeted inhibitors and immunotherapies, have shown limited success in recurrent GBM [9,10]. To date, one of the best predictors of patient survival is the ability to achieve complete tumor resection.

Historically, GBM was classified based on histological features that were also used to predict patient survival [11]]. However, advancements in molecular biology have revealed a more intricate picture, including genetic alterations in *TP53*, *PTEN*, *EGFR*, and *IDH1* [12,13], chromosomal aberrations [14], and transcriptional profiles [13,15], later documented in a large cohort by The Cancer Genome Atlas [16]. In 2010, Verhaak et al. [17] refined the classification of GBM subtypes by integrating various types of genomic data and defining: **(1) Classical Subtype** characterized by frequent *EGFR* amplification, which is associated with high cellular proliferation, aggressive behavior, and poor prognosis. Tumor cells show infiltration into healthy adjacent tissue and an inflammatory response. **(2) Mesenchymal Subtype** characterized by common alterations in *NF1* and *PDGFRA*, which result in a more invasive phenotype with increased expression of mesenchymal markers. It is associated with poorer outcomes and resistance to certain therapies; **(3) Proneural Subtype** harboring *IDH1* gene mutations, which is often linked to better survival rates compared to the classical and mesenchymal subtypes. Currently, according to WHO classification from 2021, these tumors would not be GBMs but grade 4 astrocytomas [18]; **(4) Neuronal subtype**, defined by characteristics similar to neuronal cells, is now understood to have resulted from contamination of the original samples with non-tumor cells [19]. This is because the traditional bulk RNA sequencing methods, while informative, have struggled to capture tissue complexity due to their inability to resolve individual cell states within tumors.

In contrast, scRNA-seq has transformed our understanding of intratumoral heterogeneity, revealing that GBM tumors consist of a spectrum of cell states rather than a single homogeneous population, representing various transcriptional states, including stem-like, proliferative, and differentiated states [8]. This intratumoral diversity reflects genetic heterogeneity, different clones within the tumor, and cellular plasticity, the capacity of cells to transition between states in response to environmental cues or therapeutic pressures. scRNA-seq has revealed the hierarchical organization of GBM, identifying a subset of tumor cells with stem-like properties that drive tumor propagation and therapy resistance. One key insight is the presence of evolving subclonal populations within GBM, particularly under therapeutic pressure, which often harbor distinct genetic mutations linked to varied transcriptional profiles and treatment resistance. Tirosh et al. 2016 [20] demonstrated that single-cell analyses can track the evolutionary trajectories of these subclones, providing real-time insights into tumor adaptation during treatment.

Through scRNA-seq, researchers have discovered that non-tumor cells within the tumor microenvironment (TME), such as immune cells, endothelial cells, and astrocytes, significantly contribute to GBM heterogeneity and play a critical role in its recurrence by supporting tumor cell survival and invasion [21]. Moreover, factors contributing to high recurrence rates in GBM include incomplete surgical resection, therapy-resistant

subpopulations, including glioma stem cells (GSCs), a pro-tumorigenic microenvironment, and adaptive tumor cell mechanisms. Recent single-cell and single-nucleus RNA sequencing studies have shown that GBM cells display significant phenotypic plasticity and can transition between cellular states. However, the impact of standard therapies (temozolomide, radiation, surgery) on cell type selection during recurrence and their effects on tumor-associated immune cells and non-malignant neuroglia remains unclear. This analysis revealed that recurrent GBMs are associated with transitioning toward a mesenchymal phenotype, driven by activator protein- 1 (AP-1) [21]. The Ap-1 complex comprises Jun (c-Jun, JunB, JunD) and Fos (c-Fos, FosB, FRA-1, FRA-2) protein families [22]. Interestingly, regulatory loci with confirmed c-Jun motifs have distinct DNA methylation patterns in GBMs as compared to benign gliomas, suggesting the contribution of DNA methylation to the c-Jun-dependent gene expression (e.g., *VIM*) [23] highlighting the role of epigenetic changes in regulating GBM-specific patterns. Additionally, in recurrent GBMs, an increased abundance of T-cells at recurrence correlated with tumor cell hypermutation (at least 20 mutations per megabase of DNA), which was a positive prognostic indicator [21]. The study of Wang and others [21] underscores the immune microenvironment of recurrent GBM following chemoradiotherapy. In this line, immunostaining identified M2 macrophages and immature dendritic cells as enriched in recurrent high-grade gliomas (HGGs), highlighting an immunosuppressive TME [24]. This transcriptomic dysregulation and immune cell infiltration in recurrent HGGs may limit the effectiveness of immunotherapies in GBM treatment.

3. GSC: drivers of the tumor heterogeneity

Neuronal activity and neurotransmitter signaling are crucial in regulating the proliferation of neural precursor cells, including oligodendrocyte precursor cells (OPCs) and neural stem cells in the subventricular zone and hippocampus [25]. During early neurodevelopment, synchronized waves of electrical activity and consequent voltage-dependent calcium transients occur in developing neural tissues and regulate both cellular and synaptic patterning [26]. The electrical activity also regulates the migration of newly generated neurons and influences axonal pathfinding and axonal targeting [27]. Furthermore, neurotransmitter signaling regulates brain organogenesis and later serves as the backbone of synaptic communication between neurons [28]. In early neurodevelopment, immature neural cells secrete neurotransmitters in a non-synaptic manner, facilitating neuronal generation [29]. Moreover, neurons communicate with neural stem cells and progenitor cells through activity-dependent paracrine factors such as brain-derived neurotrophic factor (BDNF) and synaptic communication [29]. Synaptic signaling is crucial for OPCs, which receive synaptic input via glutamatergic (calcium-permeable AMPA receptor-mediated) and GABAergic (GABAA receptor-mediated) neuron-to-glial synapses. These neuron-to-OPC synapses play a key role in the generating and remodeling of myelin [30].

Neftel et al. 2019 [8] identified four GBM transcriptional states resembling canonical neurodevelopmental cell types: neural-progenitor-like (NPC-like), oligodendrocyte-progenitor-like (OPC-like), astrocyte-like (AC-like), and mesenchymal-like (MES-like). Notably, cells within a single tumor can switch between these states, driven by various genetic and microenvironmental factors. This plasticity complicates therapeutic targeting and highlights the challenges in developing effective treatments for this aggressive tumor. GSCs, a subpopulation of GBM cells, contribute to this heterogeneity, resulting from their high plasticity, sustained self-renewal, persistent proliferation, and tumor initiation. Additionally, they possess

an intrinsic ability to invade the brain parenchyma, evade the immune system, adapt to microenvironmental pressures, and promote angiogenesis [31]. GSCs play a key role in driving the phenotypic diversity and plasticity of GBM, which is strongly influenced by the tumor microenvironment [32]. GSCs are classified as proneural GSCs (PN GSCs) or mesenchymal GSCs (MES GSCs) based on distinct gene signatures (for a review see [33]). In terms of metabolic patterns, PN GSCs exhibit higher OXPHOS activity, whereas MES GSCs show increased glycolytic metabolism, although they retain the ability to use OXPHOS when necessary [34,35]. PN GSCs tend to reside in the perivascular niche, whereas MES GSCs are mainly located in hypoxic/necrotic regions [33]. PN GSCs exhibit high growth rates and promote tumor angiogenesis [36], while MES GSCs display strong invasive abilities, leading to aggressive tumor growth [37] (Fig.1). However, it is generally more challenging for MES GSCs to generate tumors than PN GSCs. This physiological difference is further underscored by distinct molecular differences in splicing profiles between the two subtypes, which impact critical processes such as cell cycle, DNA repair, splicing, and cilium formation. Additionally, the expression of long non-coding RNA differs between PN and MES GSCs, and specific long non-coding RNAs have been linked to patient prognosis (for a review see [33]). Moreover, patient-derived glioma sphere cultures (enriched for GSCs) resembling either PN GSC or MES GSC transcriptional subtypes, show significant differences in their biological characteristics [38].

Interestingly, a subset of the PN GSCs can undergo transformation into MES GSCs, a phenomenon known as proneural-to-mesenchymal transition (PMT). For instance, TNF-α and NF-κB activation have been shown to enrich CD44+ MES GSC subpopulations and promote radioresistant phenotype [38]. Additionally, temozolomide treatment has been shown to induce the transformation of PN GSCs into MES GSCs [39],(Fig.1)]. Therefore, PN and MES GSCs manifest different sensitivity to radiotherapy or chemotherapy.

Neuronal activity also promotes PMT of GSCs in an exosome-mediated manner. Molecularly, neuronal activation leads to increased levels of miR-184-3p in neuron-derived exosomes, which are taken up by GSCs. This process reduces the mRNA N6-methyladenosine (m6A) levels by inhibiting RBM15 expression. RBM15 deficiency results in decreased m^6A modification of *DLG3* mRNA, subsequently inducing GSCs PMT by activating the STAT3 pathway. Notably, the loss of miR-184-3p in cortical neurons significantly reduces GSC xenograft growth, even when neurons are activated [40]. Further insights were obtained using human neocortical tissue sections inoculated with patient-derived primary glioma stem-like cells into human and rodent host environments, differing in age and sex of the neocortical donors. This study revealed that the single-cell composition of inoculated cells varied significantly depending on the source of the host tissue, suggesting that specific neuronal environments promote distinct transcriptomic programs in GSCs [41]. These findings underscore the role of neuronal environments in fostering GBM progression through the PMT induction of GSCs.

4. GBM subtypes and neurons

GBM tumors originate from glial/neuronal progenitor cells. In normal physiology, progenitor cells exit their quiescent state to differentiate into new populations of glial and neuronal cells [42]. In contrast, GBM cells continue proliferating and dynamically shift their identities toward different progenitor and stem-like cells, exhibiting remarkable plasticity. As

previously discussed [8], four cellular states were defined within GBM, highlighting this tumor's complexity and adaptability. OPC/NPC-like states were observed to be more proliferative than MES/AC-like, mixed populations of different cell states were observed more frequently between AC/MES, NPC/OPC, AC/OPC, than between AC/NPC, MES/NPC, and MES/OPC states, suggesting that there is a preference or hierarchy of transitions [8]. Interestingly, a mouse xenograft cell barcoding experiment showed that one cell with one cellular state can give rise to cells with all four defined cellular states. We do not fully understand how this plasticity is maintained, but several observations point toward potential conclusions. It was recently shown that the transcriptomic profile of GSCs in culture was relatively independent of the genetic background, possibly relying more on epigenetic control and/or the developmental stage of the cell of origin [43]. It is well established that GBM cells show remarkable heterogeneity and plasticity in their DNA methylation profiles [44,45]. However, the precise mechanisms by which cellular state transitions occur via epigenetic processes remain unclear. According to R. Chaligne et al. 2021 [44], the transition between more stem-like (OPC/NPC-like) and more differentiated (MES/AC-like) states may be partly regulated by DNA methylation, particularly at PRC2 binding sites. Nevertheless, transitions between more closely related states (MES<->AC or OPC<->NPC) are unlikely to be regulated by DNA methylation and may instead be influenced by interactions with the microenvironment. OPC/NPC cellular states showed promoter hypomethylation of known PCR2 targets, such as FOXD2, GATA6, FOXL1, POU4F2, FGF5, FGF3, CDX2, HOXD8, ESPN, GABRA4 in comparison to MES/AC cellular states. This observation strongly suggests that the PRC2 complex may be essential in cellular state switching [44]. The maintenance of PRC2 targets in a hypomethylated state is similarly observed in physiological development and is believed to be a crucial mechanism for preserving stemness potential. Authors demonstrated that promoters of genes targeted by PRC2 repressive complex in OPC/NPC states exhibit bivalent chromatin marks, characterized by the presence of repressive H3K27me3 and the activating H3K4me3 chromatin marks and hypomethylated DNA. This suggests that these genes are poised for activating; removal of the H3K27me3 repressive mark may lead to efficient gene activation [44].

Stem-like GBM cell states (OPC/NPC) are highly enriched in open chromatin regions at transcription factors ChIP-seq sites such as NF-YB, NF-YA, YY1, SP1, CHD1/2, and components of the PRC2 complex, including SUZ12 and EZH2 [44]. On the other hand, MES/AC-like states were found to have the most enriched open chromatin regions at ChIP-seq sites of some AP-1 complex transcription factors: FOSL2, JUND, FOSL1, c-FOS, and STAT3. SUZ12 and EZH2 were among the less enriched transcription factors, further reinforcing the idea that the PRC2 complex plays a crucial role in regulating the transition between OPC/NPC and MES/AC states.

Recent advances in spatial methods have led to the identification of novel cell states along with insights into their spatial organization. In a recent work [46], seven malignant, spatially resolved cellular states were characterized, partially overlapping with the Neftel 2019 classification: AC, MES, MES-Ast (astrocytic-like mesenchymal), NPC, OPC, Chrom. Reg (characterized by a strong expression of chromatin regulator genes) and Prolif Metab (enriched for proliferation and metabolism-related genes). Additionally, six normal brain cellular states were identified: Neuron, Vasc (vasculature enriched), Mac (macrophage rich), Inflammatory-Mac (inflammatory macrophage rich), Oligo (with oligodendrocyte markers), Reactive-Ast (expressing reactive astrocytes markers). Moreover, higher-order organizational units were defined, including hypoxic/necrotic niches, hypoxia-adjacent regions,

angiogenesis/immune hubs, neurodevelopmental GBM states, and infiltrated brain regions [46].

An important question in GBM research is the relationship between various GBM cellular states and neurons. Exciting new insights from emerging spatial transcriptomic and proteomic methods have revealed that neurons and MES-Ast (astrocytic-like MES-like state) are spatially distant and appear to repel each other [46]. A similar observation was made for mesenchymal hypoxic state and neurons. In contrast, NPC-like cells were found to be coupled with neurons, suggesting a possible metabolic or mechanistic dependency. The authors propose that this NPC-like and neuron coupling in infiltrative tumor areas mimics normal brain development, where neuronal progenitor cells (NPCs) migrate toward developing neurons. Similarly, malignant OPC-like cells were found to associate with vascular-rich regions (Vasc), resembling the migration of normal OPCs along the brain's endothelium [46].

5. GBM recurrence under therapy pressure

Tumor heterogeneity plays a critical role in GBM recurrence as it contributes to: **(a)** residual disease: small subpopulations of resistant tumor cells often survive even aggressive treatment, leading to recurrence that is typically more resistant to subsequent therapies [47]; **(b)** local invasion: GBM cells infiltrate surrounding brain tissue, preventing complete surgical resection and facilitating recurrence [48]; **(c)** escape strategies: wide range of molecular and cellular programs activated in response to therapy or immune responses. Single-cell analyses have identified markers associated with treatment resistance, including elevated expression of genes linked to epithelial-mesenchymal transition (EMT) and stemness [7,8,49,50]. Identifying these resistant cell subpopulations is crucial for understanding and overcoming recurrence.

Wu et al. [51] found in their single-cell resolution study the upregulation of stemness-related and cell-cycle-related genes upon GBM recurrence. They also observed reduced proportions of microglia and increased expression of vascular endothelial growth factor A (VEGF-A), and increased blood-brain barrier (BBB) permeability, as indicated by BBB markers, between initial vs recurrent GBM tissues. Additionally, Leblanc et al. [52] studied recurrent GBM at a single-cell level and found that the genetic differences between primary and recurrent tumors were smaller than between patients but larger than within a patient.

Moreover, GBM cells have a remarkable ability to adapt to therapeutic pressures. Several signaling pathways become upregulated in response to treatment, promoting survival and resistance. For instance, epidermal growth factor receptor (EGFR) amplification and mutations are common in GBM, driving both tumor proliferation and therapy resistance [53–55]. Similarly, the PI3K/AKT/mTOR pathway, involved in cell survival and metabolism, is often activated in recurrent GBM, contributing to resistance to radiation and chemotherapy [56]. This adaptive rewiring of signaling networks complicates long-term therapeutic responses.

The infiltrative nature of recurrent GBM limits the efficacy of delivery of many small and large molecules into the brain. Moreover, the BBB is a major obstacle, restricting the delivery of systemic treatments like chemotherapy and small-molecule inhibitors. Some tumor areas with active angiogenesis may have a leaky BBB, but in infiltrative regions, the intact BBB blocks drug penetration, leading to the failure of systemic therapies [57]. GBM is known for its highly immunosuppressive microenvironment, which hampers the efficacy of immunotherapies such as checkpoint inhibitors. The presence of regulatory T-cells, myeloid-derived suppressor cells, and tumor-associated macrophages creates an environment that

limits anti-tumor immune responses [58]. As a result, immune evasion significantly contributes to recurrence, and overcoming this barrier is a major focus of current research efforts.

6. Spatial organization and neurons

GBMs are composed of diverse cell types, including tumor, glial, immune, and endothelial cells. This heterogeneity is not only cellular but also spatial, resulting in regions within the tumor with distinct characteristics, such as core and peripheral regions, including the peritumoral brain zone (PBZ) bordering the tumor tissue [59]. GBM cells are known for their infiltrative growth along white matter tracts. Their spatial organization is not random but influenced by the neural tissue architecture. GBM cells can interact with neurons through direct contact or by releasing signaling molecules. The neuronal response to these signals can promote tumor growth, invasiveness, and immune evasion [59]. Tumor cells can exploit neuronal pathways to promote their survival and proliferation by secreting factors that compromise neuronal health or alter synaptic function, potentially creating a tumor-supportive niche. Andrieux et al. 2023 [60] used 5-aminolevulinic acid (5ALA) during neurosurgical resection to visualize infiltrative GBM. Results revealed regional and cell-type specific molecular subtype heterogeneity. Infiltrative 5ALA+ cells at the invasive margin exhibited mesenchymal, wound response, and glycolytic profiles, distinct from the tumor core. Garcia-Diaz et al. 2023 [61] compared matched bulk and margin cell populations in GBM mouse models. Despite genetic mutations, tumors converged on common neural-like states. In the tumor core, injury-like processes from immune infiltration created low-proliferative injured neural progenitor-like cells and dormant GBM cells triggered by interferon signaling in the T-cell niche.

In contrast, the immune-cold margin microenvironment favored developmental-like trajectories, resulting in the differentiation of cells toward invasive astrocyte-like phenotypes. Zheng et al. 2023 [62] examined how spatial cellular organization influences glioblastoma prognosis, particularly focusing on intra-tumoral heterogeneity and cell-state plasticity in therapy resistance. Their study found that poor prognosis is associated with a higher presence of tumor cells expressing a hypoxia-induced transcriptional program, as well as clustering of astrocyte-like tumor cells, which correlated with worse outcomes. Using deep learning models on single-cell RNA-seq and spatial transcriptomics data, they analyzed 40 million tissue spots from 410 patients. Validation through deep learning models on histology images confirmed the presence of survival-associated regional gene expression programs in spatial transcriptomics data.

7. Hypoxia as a spatially organizing force in GBM

Histopathologists have long noted hypoxia in classical immunochemistry slides in patients with GBM. However, there has been ongoing debate about whether it should be viewed primarily as a consequence of the tumor's rapid proliferation or as a significant driver of malignancy. While hypoxia resulting from aggressive growth is well-established, increasing evidence supports its role as a malignancy driver. Recent studies have concluded that a poor prognosis in GBMs is associated with a higher proportion of tumor cells expressing a hypoxia-induced transcriptional program, underscoring its potential impact on tumor progression [62].

In another recent work, VM Ravi et al. [41] identified five distinct spatial transcriptional programs: reactive immune, radial glia, spatial OPC, neural development, and reactive

hypoxia. The reactive hypoxia program exhibited the strongest overlap with the MES-like cellular states as defined by Neftel et al. in 2019, especially with the MES2-like state. Integrating spatial transcriptomic data with histological examinations showed significant enrichment of the reactive hypoxia program, which was observed around necrotic tissue regions. Copy number alterations (CNAs) analysis revealed a substantial accumulation of CNAs in the hypoxia reactive regions of tissue slides. This observation led to the hypothesis that the hypoxic niche of the tumor may serve as the source of *de novo* genomic alterations crucial for tumor evolution. Additionally, the hypoxic core of the tumor was defined as quiescent, and the radial glia migration signature was found to be absent within the hypoxic region but abundant around it, suggesting that hypoxia is a strong trigger of migration.

Recent research suggests hypoxia should be considered a spatially organizing force in GBM. Through extensive spatial transcriptomic and proteomic analyses, the authors developed a network graph consisting of five layers [46]. The first layer represents the hypoxic niche, which includes MES-Hyp (hypoxic MES-like cells). The second layer, termed the hypoxia-adjacent layer, comprises inflammatory macrophages (Inflammatory Mac), astrocytic-like mesenchymal (MES-Ast), and mesenchymal-like (MES) cells. The third layer is the angiogenesis immune-hub, containing T-cell, B-cell, a vasculature signature (Vasc), a macrophage signature (Mac), and proliferation and metabolism gene signatures (Prolif-Metab). The fourth layer encompasses neurodevelopmental GBM states, which include OPC, AC and NPC. Finally, the fifth layer consists of normal brain cells, including oligodendrocytes, reactive astrocytes, and neurons (see Figure 2). This multilayer organization suggests that deeper layers contain niches that are less accessible to drugs or immune cells, rendering them less sensitive to certain therapies [46]. As expected, the hypoxic/necrotic niche, enriched with hypoxic mesenchymal cells, is devoid of neuronal cells.

8. Shift of GBM phenotype in response to neuronal activity: invasion or proliferation?

Neuronal-cancer interactions are an indispensable element of GBM pathology. The nervous system can influence oncogenesis, tumor growth, invasion, metastasis, treatment resistance, stimulation of tumor-promoting inflammation, and the suppression of anti-cancer immunity [25]. Brain tumor cells themselves show multiple neural and neurodevelopmental features, including network-like structures that facilitate their spread. GBM cells invade the brain with neuronal mechanisms, migrating through perivascular spaces in an amoeboid-like mode. To navigate and colonize the brain, glioma cells extend ultra-long, neurite-like membrane protrusions called tumor microtubes (TMs), which allow them to scan the brain microenvironment, invade surrounding tissue, and colonize it by proliferation [63]. TMs, driven by the neurodevelopmental factors TTYH1 and GAP43, recapitulate many morphological, molecular, and functional aspects of neurites, specialized neuronal structures formed during development [64]. GAP43 is relevant for all known TM functions, including organelle and vesicle transport, intercellular calcium wave propagation, cell migration, and the electrical and synaptic integration of glioma into neural circuits [65], while TTYH1 is primarily involved in promoting the invasive and proliferative capacities of TMs bearing cells [66]. GBM cells with one to two TMs typically exhibit OPC/NPC-like states that resemble neural developmental states, while cells with multiple TMs display AC/MES-like states. OPC- and NPC-like cell states located at the invasive front are especially proliferative, leading to rapid and efficient dissemination throughout the brain, not only by invasion but also by proliferation along the route [63,67]. The integration of glioblastoma cells into the TM-connected network is a major

mechanism of therapy resistance in this disease. Processes such as damage repair, resistance to treatment, and adaptations to surgery, radiotherapy, and chemotherapy enhance glioblastoma cell connectivity through a denser TM-connected network, and elevated Ca2+ activity, possibly driven by pacemaker-like cells, contribute to increased resistance [68]. In a pacemaker-like manner, periodic network hub cells generate autonomous rhythmic Ca2+ activity via expression of the Ca2+-dependent potassium channel KCa3.1 (or KCNN4). The resulting Ca2+ signals activate the tumor-promoting nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, involved in the promotion of mesenchymal transition and proliferation and survival, respectively. Notably, these rare pacemaker-like cells, identified by high KCa3.1 expression, are strongly enriched in the MES-like tumor cell subpopulation. Genetic or pharmacological interference with the potassium channel KCa3.1 led to a marked reduction of tumor cell viability within the entire network, reduced tumor growth in mice, and extended animal survival. Targeting KCa3.1 potassium channels disrupts this cell population, offering a promising therapeutic approach [69].

Unconnected GBM cells are able to establish neuro-gliomal synapses in xenograft models [70]. Despite the presence of neuro-gliomal synapses, the authors did not observe action potentials in GBM cells in any of the models tested, indicating that they are not electrically excitable. However, GBM cells displayed increased frequencies of calcium transient and synchronized calcium activity patterns, likely mediated through AMPA receptors (AMPARs). Calcium signaling via AMPARs appears to be involved in GBM cell migration, as perturbing AMPAR signaling reduced invasion. Notably, *AMPAR* gene expression was enriched at the tumor rim compared to the core, supporting the role of neuro-gliomal synapses in promoting tumor invasion. This discovery led to preclinical testing of an AMPAR-inhibiting antiepileptic drug in xenograft models, which showed initial promise as a potential therapeutic strategy [71].

Neuronal activity-dependent paracrine signaling is critical in promoting glioma proliferation and growth. Key signaling molecules, such as neuroligin-3 (NLGN3), brain-derived neurotrophic factor (BDNF), and glucose-regulated protein 78 (GRP78), are secreted by neurons in response to activity and actively stimulate tumor progression. The release of NLGN3, mediated by the metalloprotease ADAM10, has been shown to significantly enhance glioma growth; inhibiting ADAM10 markedly reduces the progression of both high- and low-grade gliomas in mouse models [72]. Additionally, NLGN3 induces a synaptogenic gene expression profile in glioma cells, suggesting its role as an upstream regulator of malignant synaptogenesis and further underscoring its significance in glioma pathogenesis [73]. Paracrine BDNF signaling further promotes synaptic connectivity between neurons and glioma cells, strengthening malignant synapses. Inhibition of TrkB, the BDNF receptor, suppresses tumor growth in glioma models without TrkB translocations, resulting in TrkB fusion proteins [74]. Similar to BDNF, nerve growth factor (NGF) promotes neuronal growth and survival by binding to TrkA, a high-affinity receptor expressed on GBM cells. TrkA activation triggers pathways that increase tumor cell proliferation and resistance to apoptosis [75]. Additionally, in the GBM mouse model, insulin-like growth factor 1 (IGF-1) has been identified as another neuronal activity-regulated molecule that mediates olfactory sensory experience-dependent initiation of high-grade gliomas in the olfactory bulb [76]. Neurons from the opposite hemisphere can also stimulate GBM formation and invasion: callosal projection neurons located in the hemisphere contralateral to primary GBM tumors promote progression and widespread infiltration through connections mediated by SEMA4F, a molecule that promotes synaptic reorganization associated with neuronal activity [77].

On the other hand, gliomas exert significant influence on the CNS and are frequently associated with seizures due to glioma-induced neuronal hyperexcitability and aberrant synaptogenesis [78]. A key contributor to this hyperexcitability is glutamate secretion through the xc-cystine-glutamate transporter system, which increases excitatory signaling in the surrounding neurons [2]. This is exacerbated by the loss of inhibitory GABAergic interneurons, caused by spontaneous seizures, tumor-released glutamate, and changes in neuronal chloride transporter expression, further tilting the balance toward excessive neuronal firing [79]. Glioma cells also promote aberrant synaptogenesis, enhancing their interaction with the CNS. For example, glioma cells harboring PIK3CA point mutations secrete glypican-3, which drives abnormal synapse formation [80]. Additionally, glioma-secreted thrombospondin-1, another synaptogenic factor, enhances functional neuronal connectivity, strengthening the interaction between the tumor and the surrounding brain tissue [81]. This pathological synaptogenesis not only exacerbates neuronal hyperexcitability but also contributes to seizure activity in patients with gliomas and brain metastases. Attempts to target glioma-neuron interactions have been made and some potential therapeutic strategies are currently tested in clinical trials, summary of these attempts can be found in this review [82].

9. Inferring glioma-neuronal intercellular communication

Recent studies highlight the bidirectional signaling between glioma and neurons, which drives tumor proliferation, invasiveness, synaptic integration, and neuronal hyperactivity. Spatial transcriptomics has revealed gene modules with differential expression between the tumor core and infiltrated brain regions, identifying genes associated with glial cell differentiation, synapse processes, and Notch signaling [67]. However, the specific neural subtypes and tumor cell populations responsible for these interactions remain poorly characterized. Single-nucleus RNA sequencing (snRNA-seq) offers a promising approach to unravel these complex interactions, potentially helping to identify the key cellular players involved. In particular, identifying ligand-receptor pairs would provide insight into signaling pathways involved in the invasion and proliferation of glioma, uncovering novel therapeutic targets.

9.1 Analysis of the glioma snRNA-seq dataset

Publicly available snRNA-seq data from grade 4 gliomas (four GBM patients) were obtained from [4] and analyzed using Seurat (v5.0.2) [83]. Our primary interest was to analyze cancer cells and two types of neurons: interneurons and excitatory neurons. Initial quality control, on the raw gene expression matrices, was based on the following criteria: 1) genes expressed in fewer than five cells were removed, and 2) cells were retained if they contained more than 200 detected genes. The filtered datasets were merged into a single gene expression matrix, combining tumor and non-tumor cells. Data normalization was performed using the NormalizeData function from Seurat. To identify biologically relevant variation, the FindVariableFeatures function was applied to select highly variable genes, enhancing computational efficiency. The ScaleData function was used to standardize gene expression by centering values to a mean of zero and scaling them to a standard deviation of one, ensuring equal contribution from all genes in downstream analyses. Finally, principal component analysis (PCA) was conducted on the scaled data using the RunPCA function to reduce dimensionality, capture maximum variance, and uncover key sources of variation for

further analysis. The FindNeighbors function was applied to the merged dataset using the first 20 PCs. The number of nearest neighbors was set to 20, influencing cluster resolution. Subsequently, FindClusters was used to perform cluster analysis on the dataset, testing multiple resolutions (0.6, 0.8, 1.0, and 1.2). Finally, the RunUMAP function was employed to perform dimensionality reduction using the first 20 PCs (Figure 3A, left panel). The cell annotation used in this analysis was conducted by the authors of the original study [4].

To minimize interpatient heterogeneity and more effectively capture signals from key senders and receivers identified in CellChat, we aimed to reduce patient-specific variation. A data integration -per patient- was performed using the anchor-based CCA integration. The original principal component reduction was used as input. Next, FindNeighbors was applied to the integrated data, using the first 20 dimensions of the CCA reduction to compute neighborhood relationships. Finally, dimensionality reduction was performed using the RunUMAP function on the integrated CCA reduction to visualize the data in two dimensions (Figure 3A, right panel). Furthermore, in order to impute missing values or unobserved gene expression, Magic was applied [80], and data for selected CellChat pathways (described in 9.1) were presented as UMAP-based feature plots (Figure 3B) and violin plots (Figure 3C). Then, in cases where more than one sender or receiver was identified, the average expression was calculated using the UCell method [84]. Finally, we focused on pathways with recognized ligands and receptors.

9.2 CellChat analysis

To characterize functional interactions between neuronal and tumor cells in the context of various signaling pathways, we implemented the CellChat analysis on the dataset. To build cell-cell communication graphs specific to glioma and neuronal compartments, the following tumor cell types were selected: **OPC** (oligodendrocyte precursor cell-like: 6774 cells, which represent 32.44% of analyzed cells), **NPC** (neural precursor cell-like: 2815 cells, 13.48%), **AC** (astrocyte-like: 3420 cells, 16.38%), **MES** (mesenchymal: 4882, 23.38%) and the two neural cell types: **InN** (interneurons: 593 cells, 2.84%), **ExN** (excitatory neurons: 2398 cells, 11.48%). Based on default CellChat v2 parameters [85], all main analyses for all possible signaling pathways were performed, and a specific metric (based on normalized cellular communication matrices) was calculated for each pathway. The metric aims to rank the signaling pathways: **(i)** maximizing the sum of communication weights from neural to tumor cells, minimizing the sum of communication weights between neural cells, and **(ii)** minimizing the sum of communication weights between tumor cells. Based on this metric, (sum_neural_out^2) / ((sum_neural_in+0.0001)^2 * (sum_neural_ext+0.0001)^2), we selected the following 12 pathways where neural-glioma cell interaction exceeded tumor cells communication: DHEA, TULP, SEMA4, DHEAS, SLITRK, IGF, EPHB, SEMA3, RA, FGF, cholesterol and testosterone. For these 12 interesting pathways, ligand-receptor interactions between neural and tumor cell types obtained significantly higher weights than other interactions. The summary of the results obtained using CellChat is presented in Supplementary Figure 1.

9.3 Importance of steroids, axon guidance proteins, and growth factors in the neuronal-GBM interaction dynamics

The analysis of potential ligand-receptor interaction using the CellChat tool reveals several important pathways that may drive glioma-neuronal crosstalk (Figure 3 and

Supplementary Figure 1). Within these pathways, steroids, axon guidance, and growth factors signaling emerge as the most represented within the top identified interactions. Here, we present the top 12 signaling pathways identified through the previously outlined analysis.

9.3.1 Steroids

Steroids and their receptors are essential regulators of diverse brain functions, including socio-sexual behaviors, aggression, neurogenesis, learning, memory, mood, and cognition. In addition to acting as the target of systemic steroids, the brain itself is capable of synthesizing neurosteroids de novo from cholesterol or circulating precursors. These neurosteroids exert rapid modulatory effects on neuronal excitability, synaptic plasticity, and behavior [86]. Dysfunctions in steroid synthesis and signaling pathways have been implicated in a range of neurological and psychiatric disorders, including anxiety, depression, autism spectrum disorders, and neurodegenerative diseases such as Alzheimer's [87]. Emerging evidence also highlights the role of steroids in neuroprotection, regulation of BBB integrity, and their potential to mitigate inflammation and oxidative stress [86], further underscoring their critical importance in brain health and disease. Steroid signaling pathways, including cholesterol, DHEA, DHEAS, and testosterone, were enriched within the strongest interactions between neurons and GBM cells in the analyzed dataset.

Cholesterol

The brain comprises about 10% of body mass, yet around 25% of body cholesterol resides in the brain, building its crucial structural component, like myelin sheets [88]. Normal glial cells, especially astrocytes, are the leading producers of cholesterol. Neurons in culture rarely synthesize cholesterol, and their cholesterol demand is met by exogenous uptake [89]. A recent study suggests macrophages engulf cholesterol-rich myelin during GBM development and transfer lipids directly to GBM cells [90]. The CellChat analysis indicates that all glioma subtypes, excitatory neurons, and interneurons contribute to communication within this pathway (Figure 3, Supplementary Figure 1). Notably, the MES glioma subtype appears to uniquely sense hydroxysteroid (17β) dehydrogenase 12 (HSD17B12) via the RAR-related orphan receptor alpha (RORα).

DHEA and DHEAS

DHAE (dehydroepiandrosterone) is the endogenous steroid hormone precursor produced by the body's adrenal glands. However, it also has various potential biological effects, acting as a neurosteroid and modulator of neurotrophic factor receptors. In GBM, DHEA promotes temozolomide (TMZ) resistance through multiple mechanisms, including activation of the LYN-AKT pathway, driving Sp1 phosphorylation, protecting DNA from damage [91], and the upregulation of CYP17A1 via Sp1-mediated DNA demethylation, leading to DHEA-mediated cytoprotection [92]. Moreover, DHEA protects glioma cells from apoptosis induced by glucose deprivation during immunostimulation with interferon-gamma and lipopolysaccharides [93]. These pathways collectively enhance glioma cell survival and treatment resistance. CellChat analysis further reveals that MES and AC glioma subtypes predominantly receive DHEA and DHEAS signals from neurons, suggesting that these subtypes exploit this signaling pathway to promote resistance and sustain survival (Figure 3, Supplementary Figure 1). Regarding neuronal activity, DHEA possesses a neurotoxic and

anti-proliferative effect, while DHEA-sulfate (DHEAS) demonstrates a slight neuroprotective effect [92]. No data concerning DHEAS's role in GBM are available.

Testosterone

Testosterone is produced mainly in Leydig cells of testes in males and ovaries in females, but it is also produced in neurons [94]. Although numerous studies have explored testosterone's role in neurogenesis and sex differences in brain development, the findings remain inconsistent [95]. A well-established effect of testosterone is its enhancement of neurogenesis, by increasing the survival of newly generated neurons [95]. Recently, it was shown that testosterone upregulates GDNF and cytokine production in glioma cells, triggering neuroinflammation. CellChat analysis shows that the AC glioma subtype detects testosterone, released by neurons and other glioma cells, via androgen receptors (Figure 3, Supplementary Figure 1). Interestingly, androgen receptor deficiency was recently shown as a positive prognostic marker in glioma [96]. Moreover, glioma exhibits a higher prevalence in males than in females, and some researchers attribute this difference to differences in hormonal production [95].

9.3.3 Growth factors

The GBM microenvironment contains numerous growth-stimulating factors and their corresponding receptors. Among the most prevalent pathways identified through CellChat analysis are fibroblast growth factor (FGF) and insulin-like growth factor (IGF) signaling, as well as retinoic acid (RA), which may regulate the expression of various growth factors, including FGF and IGF.

Insulin-Like Growth Factor 1

The CellChat analysis suggests that interneurons may secrete Insulin-Like Growth Factor 1 (IGF1), which is sensed by all glioma subtypes through the IGF1 receptor (IGF1R) (Figure 3, Supplementary Figure 1). Under normal conditions, IGF1 is highly expressed in neurons but minimally in other brain cell types, while IGF1R is widely expressed in most cells, particularly in endothelial cells and astrocytes (https://brainrnaseq.org/), which is also observed in the analyzed dataset (Figure 3). Aberrant IGF signaling is observed in adult GBM, where IGF-IR and IGF-IIR are overexpressed compared to normal brain tissue. IGF-IR staining positivity has been identified as an independent prognostic factor associated with poorer prognosis [97]. Due to conflicting reports on the IGF pathway's role in promoting cell proliferation and migration via modulation of the PI3K/AKT pathway [98], further research is needed to clarify its role in GBM. Our results suggest that neurons are a key source of IGF1 for GBM cells.

Fibroblast growth factor

The FGF signaling is well described in gliomas, and FGFR often bears mutations, mainly in the tyrosine kinase domains [99]. However, our analysis identified specific FGF9 signaling interactions between interneurons and the MES and AC subtypes through fibroblast growth factor receptors 1, 2, and 3 (FGFR1, FGFR2, FGFR3) (Figure 3, Supplementary Figure 1). Fibroblast growth factor-9 inhibits astrocyte differentiation of adult mouse neural progenitor cells [100]. Aberrant FGF9 signaling may be a crucial inhibitor of GBM cell differentiation and fuel GBM growth.

Retinoic acid

The ability of retinoic acid (RA) to induce post-mitotic neural phenotypes in various stem cells *in vitro* provided early evidence of its role in the switch between proliferation and differentiation. RA is crucial for regulating the cell cycle to halt proliferation; loss of RA signaling is linked to dedifferentiation and cancer development [101]. During differentiation, RA signaling switches from CRABP-II/RAR to FABP5/PPARβ/δ. The switch is driven by the transient up-regulation of RARβ and the CRABP-II/FABP5 ratio. Both RA pathways are involved in neuronal differentiation [102]. Although RA signaling was among the top interactions identified between neurons and GBM in CellChat analysis, the specific ligands or receptors involved could not be determined.

9.3.4 Axon guidance signaling

Recently, accumulating evidence points to the emerging role of axon guidance molecules in glioma progression [103]. Indeed, the results of CellChat analysis indicated many signaling pathways involved in the neurite growth process, including ephrins, semaphorins, and SLITRK pathways. Limited data found in the literature point to their role in glioma invasion while inhibiting proliferation [104,105].

EPHB

The CellChat analysis suggests that excitatory neurons may primarily secrete Ephrin-B2 (EFNB2), which can be sensed by all glioma subtypes through the ephrin type-A receptor (EPHA4 - AC subtype) and ephrin type-B receptor 1 (EPHB1 - AC, OPC and NPC subtype) (Figure 3, Supplementary Figure 1). Eph receptors and ephrins, the largest subfamily of the receptor tyrosine kinase (RTK), play critical roles in developmental processes. Ephrins are membrane-bound proteins: class A ephrins are attached to the membrane via a glycosylphosphatidylinositol (GPI) anchor, and class B ephrins are transmembrane [106]. Eph receptors and ephrins interact to regulate cell-cell communication, cytoskeletal dynamics, cell migration, and changes in cellular morphology and behavior. In humans, loss of Reelin, a protein involved in axonal guidance, synaptogenesis, and dendritic spine formation [107], leads to lissencephaly and is linked to other neurological disorders like epilepsy, schizophrenia, and Alzheimer's [106]. It was shown that the neuronal guidance cues ephrin-B proteins are essential for Reelin signaling during the development of laminated structures in the brain [108]. Eph-ephrin interactions at synapses regulate cytoskeletal dynamics [109] and, in stroke, EphB2 binding to astrocytic ephrin-B ligands activates cytokine expression via the MAPK pathway [110]. Ephrin-B2 plays a dual role in glioma progression by promoting cell migration and invasion, enhancing tumor growth through extensive proliferation when knocked down [104,105], highlighting its importance in glioma dissemination.

SEMA4

The interplay between glioma cells and neurons is heavily influenced by the semaphorin signaling axis, particularly involving semaphorin 4D (SEMA4D) and its receptor Plexin-B1. SEMA4D is notably upregulated in neurons during the progression of neurodegenerative diseases like Alzheimer's and Huntington's disease, where it induces reactive astrocytes [111]. Blocking SEMA4D has been shown to reduce GABAergic synapse loss and improve cognitive functions [111]. SEMA4D-Plexin-B1 signaling regulates inhibitory

synapse development [112] and is associated with the pathological grade of glioma [113]. According to CellChat results, in gliomas, Plexin-B1 is primarily expressed in OPC-like and AC-like states, while excitatory neurons express high levels of SEMA4D (Figure 3, Supplementary Figure 1), suggesting that glioma cells may exploit neuronal SEMA4D to drive tumor progression and disrupt synaptic functions.

SLITRK

SLITRK proteins are highly expressed in the developing nervous system of vertebrates, where they regulate neurite outgrowth and promote synaptogenesis; however, the expression and function of Slitrk protein members differ. SLIT and NTRK-like protein-5 (SliTrk5) may be involved in the pathogenesis of CNS diseases, particularly in gliomas, where its expression is upregulated and correlates with tumor grade [114]. The CellChat analysis suggests that both interneurons and excitatory neurons may secrete SLITRK5, which all four glioma subtypes could sense through the receptor-type tyrosine-protein phosphatase S (PTPRS) (Figure 3, Supplementary Figure 1).

SEMA3

Class-3 semaphorins (Sema3) are axon guidance molecules with critical roles in GBM, semaphorin 3A (SEMA3A) and semaphorin 3D (SEMA3D) being most highlighted in our CellChat analysis. Neuropilin-1 (NRP1), a key receptor for Sema3, is essential for GBM cell migration [115]. Sema3A promotes GBM clonogenic growth while inhibiting GSC proliferation and inducing invasion [116,117]. Sema3D inhibits angiogenesis, prolonging survival in GBM mouse models [118]. Sema3D overexpression activates appetite inhibiting pro-opiomelanocortin neurons in the hypothalamus, suppressing appetite, and promoting catabolic metabolism, potentially contributing to cancer cachexia [119]. CellChat analysis suggests that excitatory neurons may secrete SEMA3A and SEMA3D as previously described in the literature [115–117], pointing to plexin A2 (PLXNA2) and plexin A4 (PLXNA4) as potential receptors present on the AC and MES glioma cells (Figure 3, Supplementary Figure 1).

9.3.4 Others

TULP

The CellChat analysis suggests that both interneurons and excitatory neurons may secrete tubby protein homolog (TUB), which could be sensed by the AC glioma subtype through the proto-oncogene tyrosine-protein kinase MER receptor (MERTK) (Figure 3, Supplementary Figure 1). Tubby and tubby-like protein 1 (Tulp1) are newly identified phagocytosis ligands that facilitate retinal pigment epithelium (RPE) and macrophage phagocytosis via the MerTK receptor [120–122]. In GBMs, MerTK is expressed by glioma-associated macrophages and microglia [123], which corresponds to our data (Figure 3B and 3C). MerTK inhibition decreases immunosuppressive GBM-associated macrophages and neoangiogenesis in the GBM microenvironment [124]. The TUB-MerTK axis has been implicated in glioma-neuron interactions, particularly in the astrocyte-like (AC-like) glioma subtype, where astrocytes phagocytize neuronal material, including synapses, apoptotic neurons, and degenerating axons [125]. Despite emerging evidence of TUB as a phagocytosis ligand, its role in neuronal cells and interaction with MerTK in GBM remains unexplored and warrants further investigation.

10. Conclusions and future directions

Our motivation to study neuronal-glioma interactions arises from their status as an understudied yet critically important area of research. Emerging evidence highlights the pivotal role of glioma-neuron interactions, particularly gliomas' ability to hijack natural neurodevelopmental cues. Glioma cells not only mirror neurodevelopmental pathways, mimicking OPC or NPC, but GBM progression also appears to rely partly on parasitic-like interactions with neurons. Understanding these interactions is essential for the development of effective therapeutic strategies. One major technical challenge in studying neuronal cells at the single-cell level is their complex morphology, which complicates the preparation of single-cell suspensions. As a result, single-nuclei approaches are often required but are less frequently employed. To bridge this gap in understanding neuron-glioma interactions at the single-cell level, we re-analyzed an existing snRNA-seq dataset to identify potential interactions and guide future research directions. Our CellChat analysis revealed that glioma-neuron interactions engage a wide range of diverse signaling pathways and mechanisms, underscoring their complexity and importance. These findings emphasize the need to further explore their role in glioma pathogenesis. Based on our analysis (Figure 4), critical avenues for future research include steroid signaling (e.g., cholesterol, testosterone, DHEA, DHEAS), growth pathways (e.g., IGF, FGF, RA), and axon guidance-specific pathways (e.g., SEMA3/4, EPHB, SLITRK). A more comprehensive understanding of these complex interactions could unveil novel therapeutic strategies targeting glioma-induced synaptic alterations and modulation of the tumor microenvironment.

Figure legends

Figure 1.

Key features distinguishing proneural GSCs (PN GSCs) from mesenchymal GSCs (MES GSCs) include growth rate, invasiveness, expressed markers, metabolic patterns, and tumorigenic potential. Factors driving the phenotypic switch between GSC subtypes are also illustrated.

Figure 2.

Schematic representation of the 5-layer organization of GBM tumor tissue, illustrating the spatial distribution of cells driven by a hypoxia gradient. The plot is adapted from the findings reported by Greenwald et al. 2024.

Figure 3.

Analysis of main neuron-glioma interaction pathways identified through CellChat analysis. A) UMAP dimensional reduction based on the first 20 PCA components for four GBM samples; left, before data integration analytical step; right, after data integration to minimize interpatient heterogeneity. B) Pairs of the UMAP-based feature plots using the integrated data. Each pair represents one pathway, highlighting the expression of the primary sender (left side of a pair) and receiver cells (right side of a pair) identified for the top 11 neuron-glioma interaction pathways. C) Expression levels of the main sender and receiver cells across all identified

glioma and non-tumor cells for the top 11 neuron-glioma interaction pathways. In cases where more than one sender or receiver was identified, a scoring gene signature was calculated using UCell and expressed as "_S" for senders or "_R" for receivers, depending on the group.

Figure 4.

Schematic summary of main neuron-glioma interactions identified from the literature and our CellChat-based analysis using a dataset of Patel KS et al. (2024).

Supplementary Figure 1.

Interaction plots from CellChat analysis show the top 12 interactions between neurons and glioma cells in the dataset of Patel KS et al. (2024). The width of the line corresponds to the strength of each interaction.

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CRediT authorship contribution statement

Marta Maleszewska: Conceptualization, Writing - Original Draft, Writing - Review & Editing; **Adrià-Jaume Roura:** Writing - Original Draft, Writing - Review & Editing, Visualization; **Michal J. Dabrowski:** Methodology, Writing - Original Draft, Writing - Review & Editing; **Michal Draminski:** Software, Formal analysis; **Bartosz Wojtas:** Conceptualization, Writing - Original Draft, Writing - Review & Editing.

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Conflict of interest

All authors declare no conflict of interest.

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Figure 1

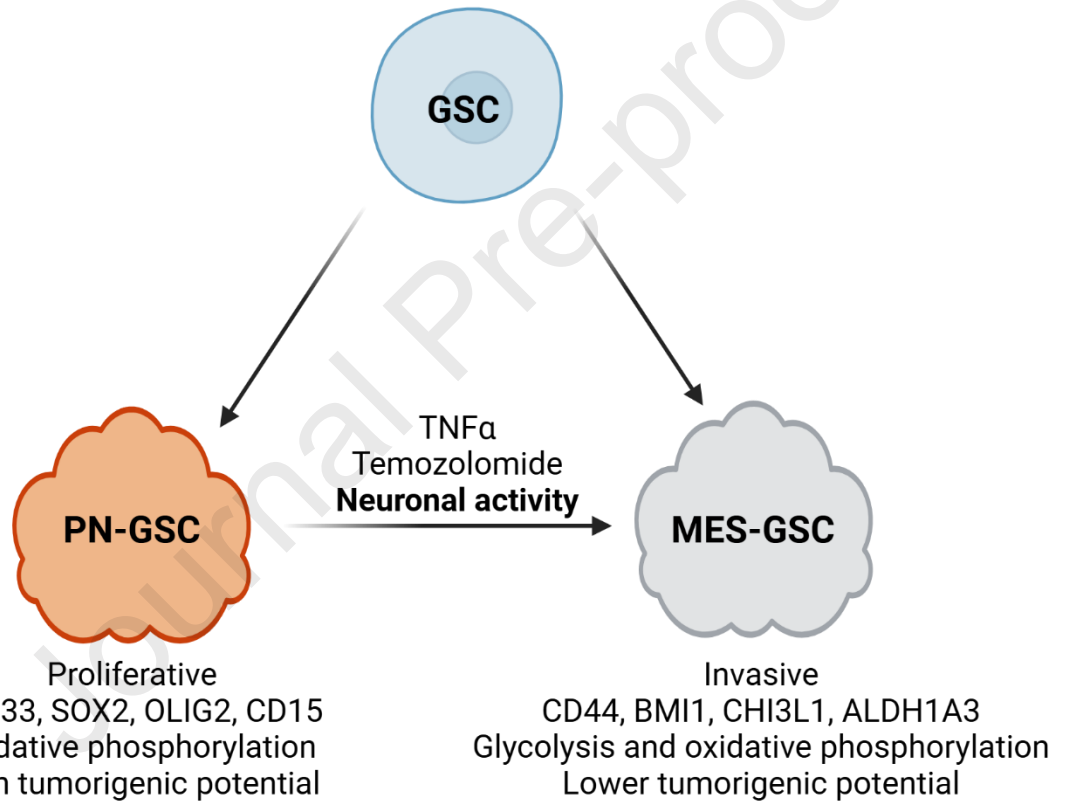


Figure 2

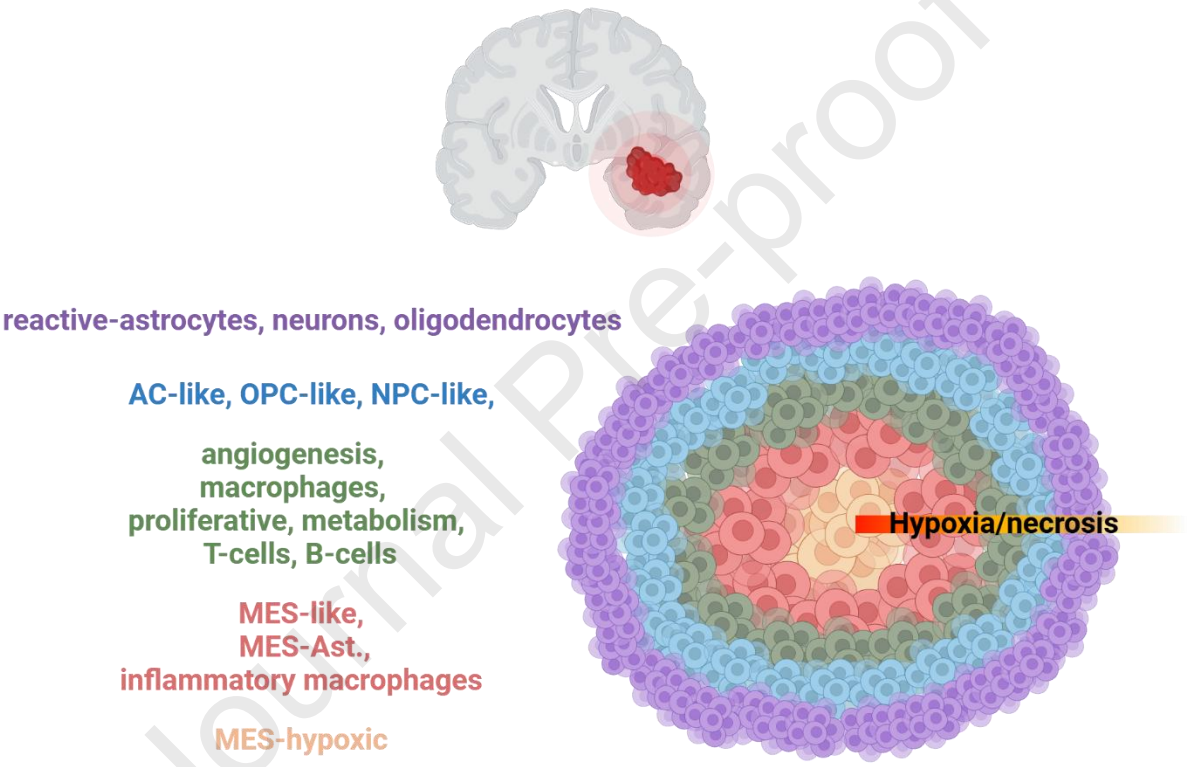
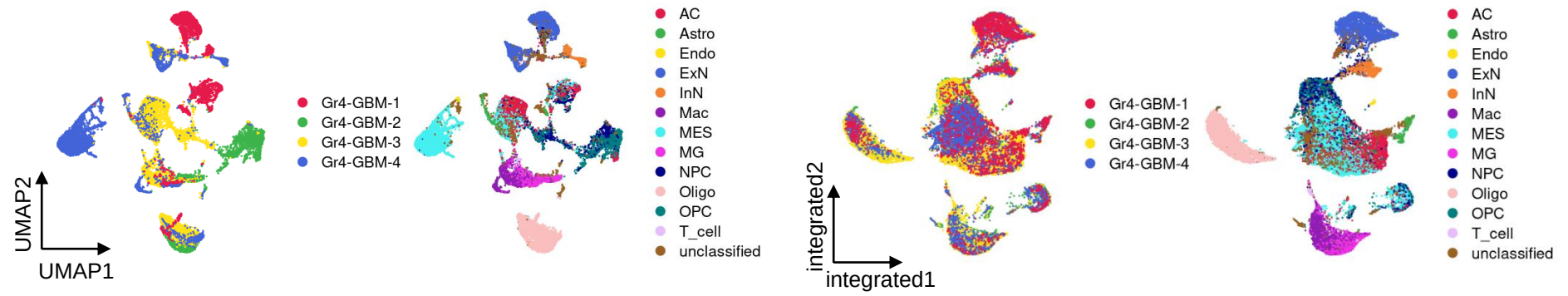
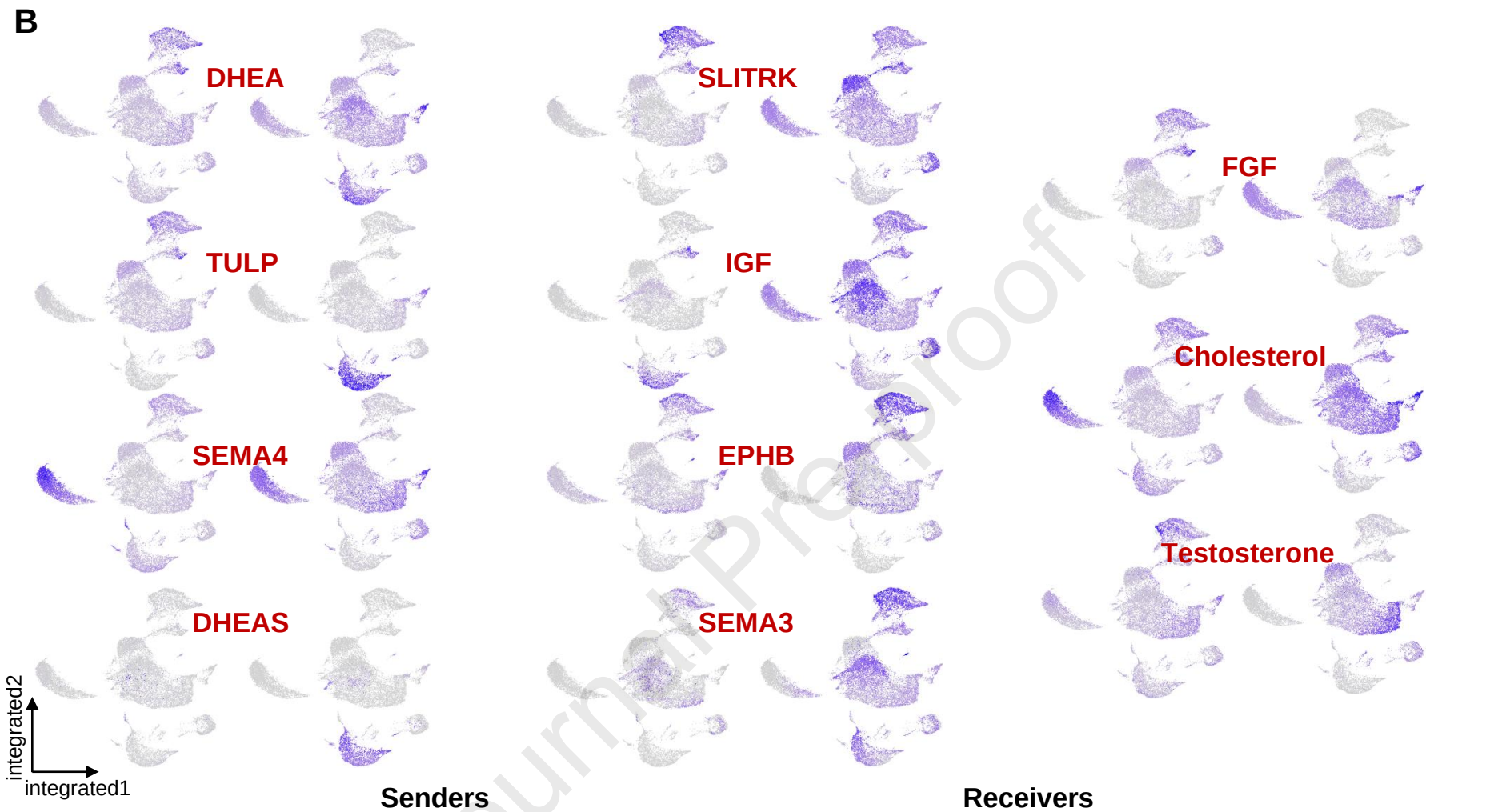


Figure 3





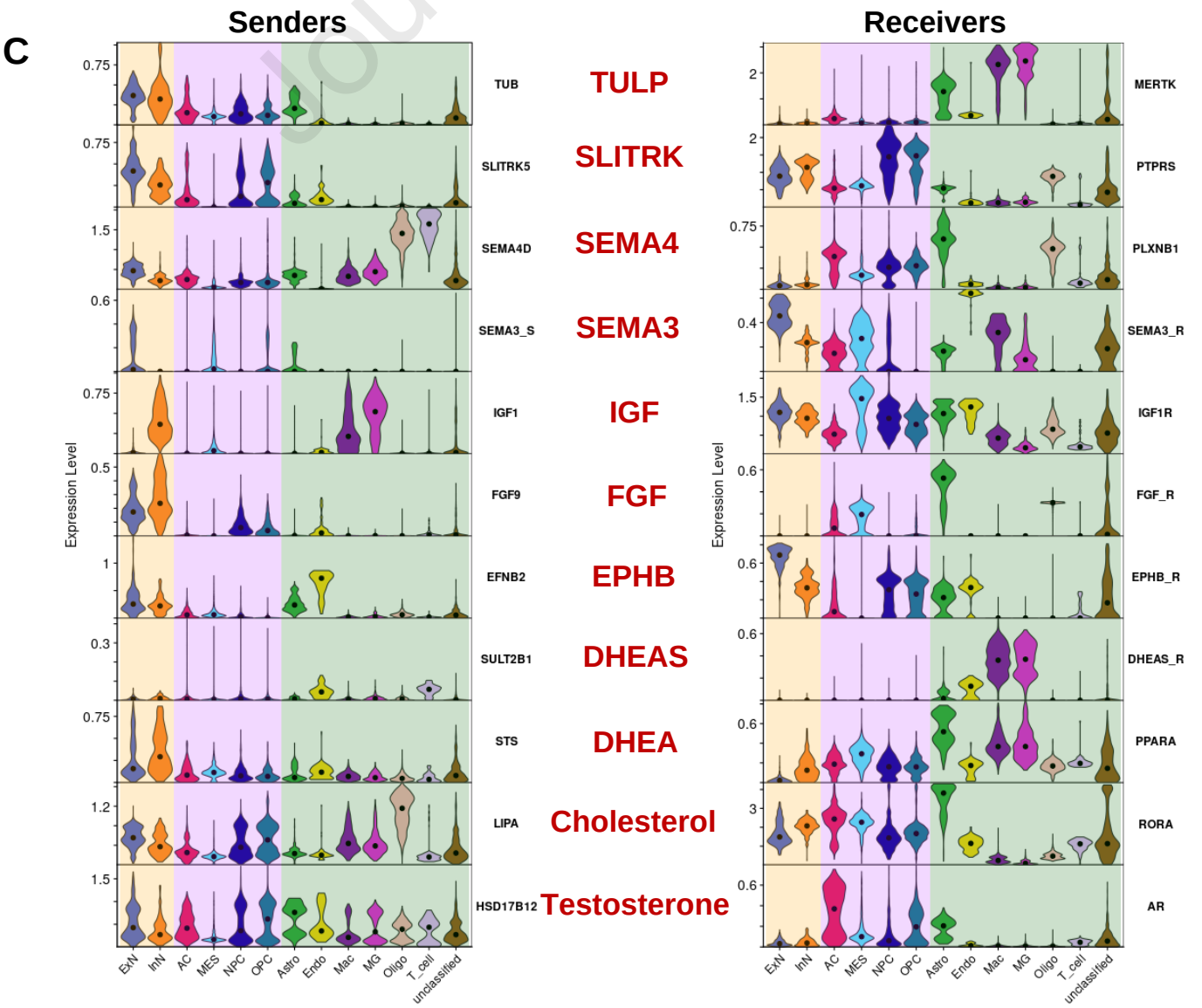
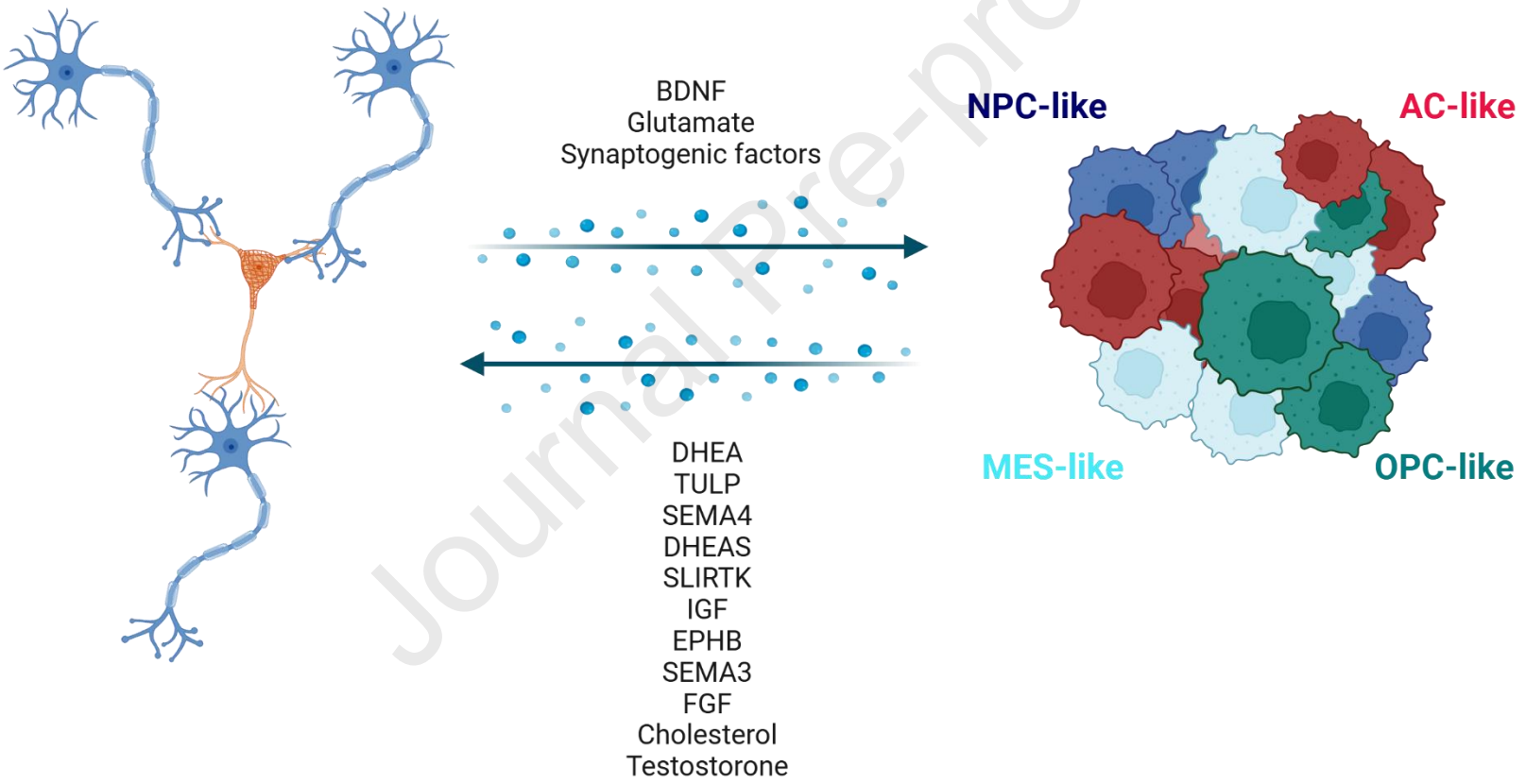


Figure 4



- GBM development mirrors neurodevelopment but exploits it for tumor progression
- snRNA-seq allows analysis of GBM - neurons interactions
- GBM interact with neurons, amplifying pro-tumor signals, such as hyperexcitability