


## Review

## Injury programs shape glioblastoma

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**Glioblastoma is the most common and aggressive primary brain cancer in adults and is almost universally fatal due to its stark therapeutic resistance. During the past decade, although survival has not substantially improved, major advances have been made in our understanding of the underlying biology. It has become clear that these devastating tumors recapitulate features of neurodevelopmental hierarchies which are influenced by the microenvironment. Emerging evidence also highlights a prominent role for injury responses in steering cellular phenotypes and contributing to tumor heterogeneity. This review highlights how the interplay between injury and neurodevelopmental programs impacts on tumor growth, invasion, and treatment resistance, and discusses potential therapeutic considerations in view of these findings.**

### The landscape of glioblastoma

**Glioblastoma** (see [Glossary](#)) is the most common and aggressive primary brain cancer in adults, and is almost universally lethal with overall survival times among the worst of any cancer. This is in part due to pronounced therapy resistance [1–3]. **Intratumoral heterogeneity** is pervasive and is often credited as the major hurdle to effective therapeutic intervention, because subpopulations of cells show different sensitivities to treatment [4–6]. One of the most prevalent patterns of transcriptional heterogeneity results from recapitulation of **neurodevelopmental lineage hierarchies** [7,8], a remnant of the neural origin of these tumors [9–11]. These lineage states exhibited by malignant cells are strongly influenced by the microenvironment in a region- and context-specific manner [6]. Recent evidence highlights a prominent role of **injury** responses as additional drivers of phenotypic heterogeneity [12–14] which result from damage caused by the growing tumor mass to the surrounding brain tissue via physical, ischemic, metabolic, neuro-inflammatory, and neurotoxic insults [15–22]. The interplay between neurodevelopmental and injury programs is emerging as an important factor impacting on treatment sensitivity [12].

In this review we describe how tissue injuries induced by developing tumors have two important consequences: (i) by eliciting injury responses in non-malignant cells, which feed back to shape tumor biology, and (ii) through the activation of similar albeit aberrant injury programs in tumor cells, which influence their malignant behavior. We explore the emerging concept that malignant cells respond to such injury cues by activating latent reactive and regenerative responses. Throughout we describe observations from both human and rodent studies. We highlight key outstanding questions and discuss how progress in our understanding of injury–tumor crosstalk may pave the way for greater insights into tumor biology, and ultimately translate to novel therapeutic applications.

### Neurodevelopmental hierarchies in glioblastoma

Single-cell analyses have demonstrated that, despite interpatient differences, intratumoral transcriptional patterns converge on a handful of hierarchically organized states that resemble normal neural lineages ([Figure 1](#)) [7,8,23]. These states are commonly referred to as oligodendrocyte progenitor cell-like (OPC-like), neural progenitor-like (NPC-like), astrocyte-like (AC-like), and

### Highlights

Glioblastoma is a deadly brain cancer comprising cells that recapitulate normal neurodevelopmental lineage hierarchies.

Increasing evidence suggests that injury responses superimpose onto these neurodevelopmental programs to fuel tumor heterogeneity and result from the growing tumor mass physically damaging the surrounding brain tissue, as well as from therapeutic intervention.

Non-malignant cells within the normal brain tissue respond to tumor-induced injury by activating reactive programs, which in turn modulate glioblastoma biology.

Tumor cells aberrantly mirror injury programs of their non-malignant counterparts by enacting latent reactive and regenerative responses.

The interplay between neurodevelopmental and injury programs has therapeutic implications, and increased understanding of the molecular basis of these programs may lead to improved treatments.

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**mesenchymal-like (MES-like)** [7]. The MES-like state, although it does not resemble a normal neurodevelopmental phenotype, does show similarities to reactive astrocytes and will be discussed in detail later. It is important to note that these states are not discrete entities; instead, tumor cells display a continuum of 'differentiation' ranging from stem-like to more differentiated states, and exhibit plasticity between lineages [7].

The presence of multilineage differentiation reflects the cellular origin of these tumors which are believed to derive mainly from neural stem cells (NSCs) or lineage-restricted progenitor cells such as OPCs [9,11,24]. In patients, low-level driver mutations have been observed in the **subventricular zone (SVZ)** of the brain, suggesting that, at least in some cases, tumors originate from this stem cell niche [9]. In support, functional studies in mice have shown that selective induction of somatic mutations in NSCs leads to tumorigenesis [10,11,25]. Although NSCs can give rise to tumors, in the normal brain they are outnumbered by OPCs, which exhibit a low level of proliferation throughout adulthood in humans [26]. As such, OPCs also represent a prime candidate as cell-of-origin for glioblastoma. Indeed, the same genetic alterations that drive tumorigenesis in NSCs also give rise to tumors when induced specifically in OPCs in mice [10]. Moreover, *p53/Nf1* deletion in NSCs has been shown to drive tumorigenesis through expansion of their OPC progeny [11]. Conversely, deletion of the same tumor suppressors in more mature cells of the neuronal lineage does not lead to tumor formation, indicating that the ability of these mutations to induce tumors declines with differentiation [27]. Although immature cell types are far more susceptible to malignant transformation, it is possible that tumors may also derive from mature cells in some circumstances. For example, *p53* deletion in combination with overexpression of oncogenic Ras can drive tumorigenesis in cortical astrocytes [28]. However, it should be noted that, although Ras signaling is frequently altered in glioblastoma, *RAS* mutations are uncommon [29].

The cell-of-origin may in part also influence the proportion of malignant cells that occupy the different lineage states within a tumor. In mice, identical tumorigenic stimuli induced in different stem/progenitor subpopulations produce markedly different glioma phenotypes, depending on the differentiation status of the transformed cell [25,30]. Tumors derived from OPCs show an enrichment for expression of oligodendrocyte-lineage genes, whereas those derived from more immature GFAP<sup>+</sup> NSCs have a greater capacity for self-renewal and shorter survival [10,25]. Although ascertaining the cell-of-origin in human glioblastoma is much more challenging, patient tumors show biases towards enrichment of transcriptional signatures associated with either an NSC or OPC cell-of-origin in mouse [30]. In addition to cell-of-origin biases, the lineage trajectory of a cell is also influenced by somatic mutations [7,13,31,32]. Common alterations such as amplification of *EGFR*, *PDGFR*, or *CDK4* can favor AC-like, OPC-like, or NPC-like states, respectively, whereas deletion of *NF1* favors a MES-like state [7,33]. However, although both cell-of-origin and genetic alterations bias cells towards a particular cell fate, genetic subclones comprise multiple phenotypes, indicating that non-genetic factors play a prominent role in determining cellular identity [7]. In this regard, different tumor microenvironments have been shown to promote stemness or drive differentiation [13,31–36] (Figure 1).

A prominent emerging feature of many of these microenvironments is the involvement of injury-associated processes which occur as a result of tumor expansion. Indeed, recent single-cell RNA sequencing (scRNA-seq) analyses of human glioblastoma found that injury-associated inflammatory and wound-response signatures account for much of the transcriptional heterogeneity in glioblastoma [12]. Furthermore, spatially separated injury-associated microenvironmental stimuli including hypoxia, immune infiltration, and white matter damage have all been implicated in steering cell fate, as determined by recent spatially resolved transcriptomic, metabolomic, and proteomic analyses, as well as by bulk RNA-seq or scRNA-seq from distinct tumor regions [13,37,38] (Figure 1). These processes will be the focus of the following sections.

## Glossary

### Damage-associated molecular patterns (DAMPs):

molecules released from damaged/dying cells after injury that are recognized by cells of the innate immune system (via PRRs); DAMPs are also known as danger-associated molecular patterns, danger signals, and alarmins.

**Dedifferentiation:** a process by which a differentiated cell reacquires stemness features.

**Glioblastoma:** this term is now formally reserved for grade IV *IDH* wild-type tumors; however, grade IV *IDH*-mutated astrocytomas were, until the 2021 reclassification by the World Health Organization, also referred to under the umbrella term of glioblastoma.

**Injury:** a complex phenomenon driven by tissue damage that involves a range of biological processes aimed at restoring tissue homeostasis. Injury encompasses a range of insults which in the context of glioblastoma include physical, ischemic, metabolic, neuroinflammatory, and neurotoxic insults.

**Intratumoral heterogeneity:** the diversity of malignant cells within a single tumor (morphological, transcriptional, genetic, epigenetic, functional). Not to be confused with intertumoral heterogeneity which is the diversity between the tumors of different patients.

**Lipopolysaccharide (LPS):** an endotoxin found on the outer membrane of Gram-negative bacteria which is commonly used (either via systemic or intracranial injection) in mouse models of neuroinflammation.

**Mesenchymal:** cells that develop into connective tissue, blood vessels, lymphatic tissue, bone, and cartilage. Mesenchymal gene-expression signatures are enriched in a subset of tumor cells commonly referred to as mesenchymal-like cells. When this signature dominates in bulk tumor samples, they are described as being of a mesenchymal subtype. This description is at odds with the neural origin of glioblastoma and has more recently been shown to overlap with that of reactive astrocytes.

### Neurodevelopmental lineage

**hierarchies:** refers to the hierarchy of cellular states in glioblastoma that recapitulate the classical hierarchy of neural stem cells at the apex which differentiate into neurons, astrocytes, and oligodendrocytes.

## Glioblastoma injures the brain

Injury is a complex phenomenon driven by tissue damage, and involves a range of biological processes aimed at restoring tissue homeostasis. Injury of brain tissue encompasses insults ranging from traumatic brain injury (TBI), which causes stretching and tearing of axons and **Wallerian degeneration** [39], to ischemia, which occurs when blood flow and oxygenation do not meet demand, leading to metabolic collapse and cell death [40]. Such insults can lead to disruption of the blood–brain barrier (BBB), death of neurons and oligodendrocytes, and the release of inflammatory molecules and/or particles, including apoptosis-associated extracellular vesicles and **damage-associated molecular patterns (DAMPs)** [41,42]. In response, microglia and astrocytes, as well as peripheral immune cells which infiltrate the injury site following breakdown of the BBB, elicit diverse inflammatory and neurotrophic programs aimed at restricting the area of injury, clearing debris, and promoting wound healing [43,44].

Similar processes are induced in the context of glioblastoma, where the growing tumor mass injures the brain by exerting physical forces on the surrounding tissue [15], and/or by inducing hypoxia/nutrient stress [16] and releasing tumor-derived factors, which include toxic levels of cytokines [17], glutamate [18], and free radicals [19] (Figure 2). In addition, standard-of-care treatments also induce forms of injury [20–22]. For example, surgical brain injury is an unavoidable aspect of tumor resection because of the nature of neurosurgical maneuvers which involve incision and electrocauterization of brain tissue [20,21]. Furthermore, tumor cell death caused by chemotherapy and radiation can impact on the function and viability of the surrounding brain tissue [22].

In the following, we first explore how normal non-neural cells respond to tumor growth, focusing on microglia/macrophages. We then focus on the reactive and regenerative injury processes of neural lineage cells (astrocytes, OPCs, NPCs) which are echoed by their malignant counterparts and can impact on proliferation, invasion, and treatment resistance.

### Microglia/macrophages

The brain is under constant surveillance by microglia, the resident macrophages of the CNS and first line of defense against insult [45,46]. In the healthy brain, microglia contribute to CNS homeostasis by secreting neurotrophic factors [47] and playing a role in synaptic pruning [48]. They express a wide repertoire of **pattern recognition receptors (PRRs)**, including Toll-like receptors (TLRs) and C lectin receptors, that allow them to sense DAMPs such as those released by dead or dying cells [49]. In glioblastoma, DAMPs are also detected by circulating monocytes which, facilitated by BBB breakdown, infiltrate the tumor where they mature to become macrophages. During tumor development, the evolving injury and progressive BBB breakdown are associated with a shift in the **tumor-associated macrophage (TAM)** population from largely microglial to a predominance of infiltrating monocytes/macrophages within the tumor mass [50–53]. Once activated, macrophages exert a combination of pro- and anti-inflammatory effects. They can contribute to a proinflammatory microenvironment by secreting tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin 1 alpha (IL-1 $\alpha$ ), complement component 1q (C1q), interleukin 1 beta (IL-1 $\beta$ ), interleukin 6 (IL-6), interleukin 12 (IL-12), and CC motif chemokine ligand 2 (CCL2), recruiting peripheral immune cells, generating reactive oxygen species (ROS) and nitric oxide (NO), and high expression of MHC class II [54,55]. Conversely, macrophages can also elicit an anti-inflammatory response and secrete interleukin 10 (IL-10) and transforming growth factor beta (TGF- $\beta$ ), as well as growth factors and neurotrophic factors such as fibroblast growth factor (FGF), colony stimulating factor 1 (CSF1), insulin-like growth factor 1 (IGF1), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophins, and glial cell-derived neurotrophic factor (GDNF) [54,55]. The complex interplay of these apparently heterogeneous response profiles differs in a region-specific manner, and analysis of scRNA-seq data from patients shows that TAMs within

### Pattern recognition receptors

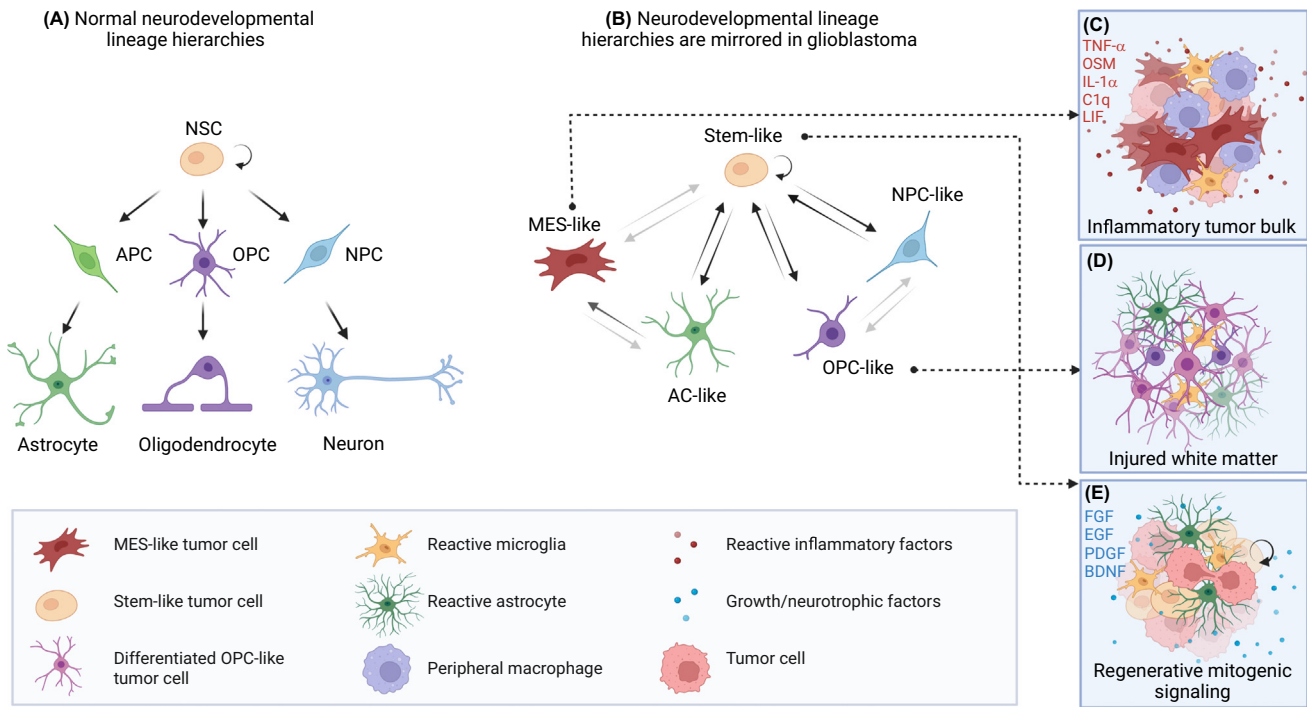
**(PRRs):** proteins on the cell membrane which recognize molecules/antigens commonly expressed by damaged cells (DAMPs) or pathogens (pathogen-associated molecular patterns).

**Proneural:** a subtype of glioblastoma tumors (together with mesenchymal and classical) which correspond to more OPC-like or neuronal-like differentiation and are commonly associated with mutations in *TP53/IDH* and amplification of *PDGFRA*.

**Subventricular zone (SVZ):** one of the neurogenic niches in the adult mammalian brain in which neural stem cells reside (another major neurogenic niche is the subgranular zone of the hippocampus).

**Tumor-associated microglia/macrophages (TAMs):** microglia/macrophages that surround/infiltrate the tumor and contribute to the tumor microenvironment.

**Wallerian degeneration:** a cell-autonomous programmed axon death pathway. Transection injuries and processes that interrupt axonal transport lead to activation of this molecular pathway, thereby triggering active degeneration of the distal axonal segment. This axon degeneration may be accompanied by a 'dying-back' pathology that culminates in neuronal death.



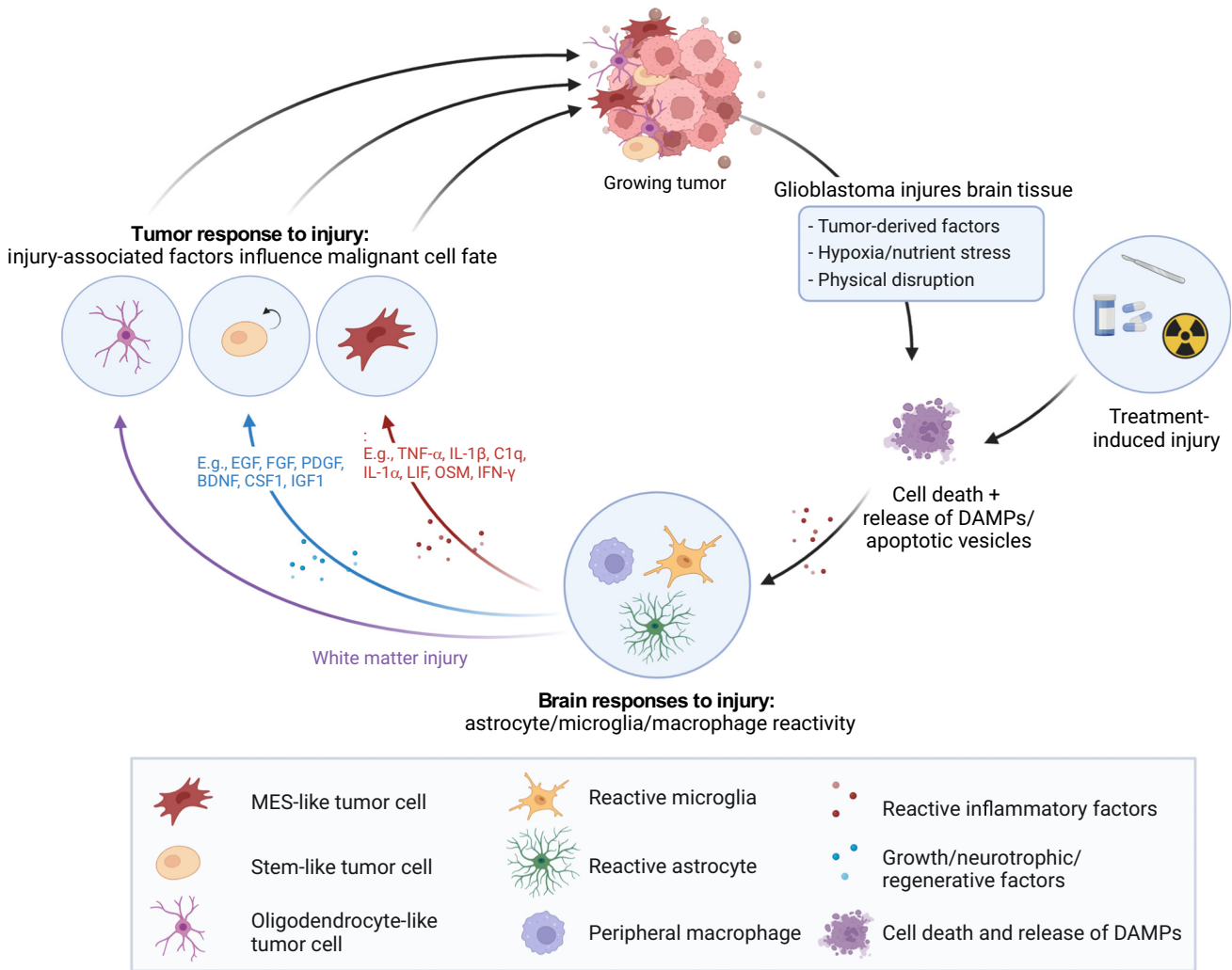
Trends in Neurosciences

**Figure 1. Intratumoral transcriptional patterns converge on hierarchically organized states that resemble normal neural lineages.** (A) In normal neurodevelopment, neural stem cells lie at the apex of a hierarchy and are able to self-renew and give rise to lineage-committed progenitors which differentiate into the normal neural cell types of the brain: astrocytes, oligodendrocytes, and neurons. (B) Glioblastoma cells mirror the normal neurodevelopmental hierarchical organization: at the apex lies self-renewing stem-like/progenitor cells which retain the ability to partially differentiate towards AC-like, OPC-like, or NPC-like cells. Differentiation is associated with a decrease in proliferation. Cells can also acquire a MES-like state which shows similarities to reactive astrocytes. Increasing evidence suggests that cell states are not terminal. Instead, cells lie within a gradient of these fates and are able to shift and dedifferentiate to more stem-like states or be in hybrid states. Black arrows indicate previously confirmed cell state shifts, whereas gray arrows indicate putative cell state shifts. (C–E) Cellular states/fates can be influenced by injury. The proinflammatory milieu of the immune-infiltrated tumor core causes a shift of tumor cells towards the MES-like cell fate (C). Tumor-induced injury to the white matter can induce differentiation of glioblastoma cells to a differentiated OPC-like fate (D) which decreases their proliferation. Conversely, neurotrophic signaling may support the stem-like fate at the apex of the hierarchy (E). Understanding how these different fates respond to treatment will be important for overcoming glioblastoma treatment resistance. Abbreviations: AC, astrocyte; APC, astrocyte progenitor cell; MES, mesenchymal; NPC, neural progenitor cell; NSC, neural stem cell; OPC, oligodendrocyte progenitor cell.

the core of the tumor, which are largely bone marrow-derived, tend towards more proinflammatory features, whereas those at the periphery, which are largely microglia-derived, tend towards more anti-inflammatory features [50–53]. Such spatial heterogeneity is consistent with recent scRNA-seq findings in the context of demyelinating injuries [56,57] and **lipopolysaccharide (LPS)** exposure (a model of neuroinflammation) [58], where even more complex spatial and temporal diversity of microglia in their resting or activated states has been described. Although TAMs have not yet been profiled with the same spatial and temporal resolution in glioblastoma, it is conceivable that the effect of TAMs on tumor biology may also be modulated by their anatomical location within the brain, duration of activation, or even sex, because sex-specific differences have been observed in glioma-activated microglia in mice [55]. What is clear is that TAMs play a central role in forming tumor niches and influencing glioblastoma cell fate by inducing reactive/mesenchymal phenotypes in neighboring tumor cells (see below).

### Reactive astrocytic processes are echoed in glioblastoma

Astrocytes respond to injury stimuli by undergoing a program of reactive astrogliosis. This reactive state was classically defined by the upregulation of GFAP; however, it is now clear that



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**Figure 2. Injury–tumor crosstalk shapes glioblastoma.** As the tumor develops, it injures the surrounding tissue via physical disruption, hypoxia/nutrient stress, and tumor-derived factors. This, together with tissue injury due to treatment, induces cell death and release of DAMPs, driving the activation of reactive cell types (astrocytes, microglia, macrophages), and the secretion of proinflammatory and neurotrophic factors which feed back to influence malignant behavior. Inflammatory signaling can induce a cell state shift towards a MES-like fate, whereas growth/neurotrophic factors can drive proliferation of a stem-like compartment. In the injured white matter, factors so far unknown can drive differentiation of OPC-like tumor cells. Abbreviations: DAMP, damage-associated molecular pattern; MES, mesenchymal; OPC, oligodendrocyte progenitor cell.

reactive astrocytes comprise a functionally heterogeneous population of cells [59]. Following injury, astrocytes can activate a diverse repertoire of molecular signaling networks which include proinflammatory, anti-inflammatory, immunosuppressive, neurotoxic, or neurotrophic responses, and are influenced in a context- and region-dependent manner [60]. Astrocytes become reactive through either direct or indirect activation. They can sense DAMPs directly through PRRs, such as TLRs and nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs), and sense mechanical stress via mechanosensitive ion channels [61,62]. Increases in extracellular ATP caused by cell death/damage induce a wave of calcium signaling between astrocyte networks that leads to ATP release from the astrocytes themselves, thereby recruiting microglia to the site of injury [62]. Astrocyte reactivity can also be induced by crosstalk with microglia/

macrophages. In the context of LPS treatment, activated microglia release TNF- $\alpha$ , IL-1 $\alpha$ , and C1q to induce a reactive proinflammatory astrocyte state which is cytotoxic to neurons and oligodendrocytes [63,64]. A similar proinflammatory astrocyte state has been identified in amyotrophic lateral sclerosis (ALS), and was associated with downregulation of genes involved in neuronal support, such as glutamate uptake [65]. Conversely, reactive astrocytes can also exert neuroprotective effects, as observed in ischemic stroke and experimental autoimmune encephalomyelitis (EAE), whereby microglial/macrophage-derived oncostatin M (OSM) was shown to promote a neuroprotective astrocyte phenotype [66]. These extracellular cues are transduced intracellularly by the transcription factors signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappa B (NF- $\kappa$ B) to elicit neuroprotective and neurotoxic states, respectively [63,67–70]. In addition to influencing inflammation, a subset of reactive astrocytes proliferate to form a physical barrier known as an astrocytic scar that restricts the site of injury, thereby limiting the spread of inflammation. This can be neuroprotective because ablation of scar-forming astrocytes leads to worse neuronal survival [71].

Astrocytes also play a central role in glioblastoma, where they have been shown to acquire a reactive state within the tumor microenvironment [68,72,73]. In the tumor bulk, transcriptional profiling has found that tumor-associated astrocytes (TAAs) acquire a progenitor- and anti-inflammatory-like state which is regulated in part by microglia, as demonstrated by depletion of microglial cells [68,72], whereas at the margin TAAs can form a peritumoral scar [73]. Thus, TAAs share similarities to reactive astrocytes that are associated with other insults to brain tissue.

The presence of neurodevelopmental hierarchies in glioblastoma poses the question of whether malignant AC-like cells enact similar reactive processes in non-malignant astrocytes. Recent evidence indicates that this might be the case; data highlighting the contribution of injury programs to tumor heterogeneity have found that the injury-associated genes expressed in glioblastoma show significant overlap with those associated with a reactive astrocyte state [12,74]. This reactive AC-like state corresponds to MES-like signatures described in the wider literature [7, 12,33,75], suggesting that the MES-like state encompasses cells which have activated latent reactive programs in response to microenvironmental stressors [37].

In a similar manner to normal astrocytes, which can be induced to a reactive state by crosstalk with microglia/macrophages, the MES-like signature is strongly associated with the presence of TAMs [6,23,31,33,72,76,77] (Figure 2). In mouse models and in patient samples, there is an enrichment of macrophages adjacent to MES-like glioblastoma cells [31]. Furthermore, recent spatial transcriptomic analysis from patient tissue demonstrates that areas enriched in myeloid populations are also enriched in both MES-like and AC-like signatures [37]. Depletion of TAMs has been shown to decrease the density of MES-like cells in a mouse model of glioblastoma, indicating that TAMs promote this reactive state in a manner similar to that observed for non-malignant astrocytes [31]. There are also further striking parallels at the molecular level whereby signaling events that underlie astrocyte reactivity also regulate the MES-like program in glioblastoma and astrocyte reactivity. In glioblastoma the MES-like program is mediated, at least in part, by TAM secreted factors – OSM, TNF- $\alpha$ , C1q, IL-1 $\alpha$ , interferon gamma (IFN- $\gamma$ ), and to a lesser extent leukemia inhibitory factor (LIF) [6, 12,31,72,76,78] – and is enforced by the transcription factors CCAAT/enhancer-binding protein beta (CEBP $\beta$ ), STAT3 [79], TAZ [80], and NF- $\kappa$ B [6].

Considering the similarities between MES-like cells and reactive astrocytes in glioblastoma, it is tempting to speculate that the MES-like state may derive from an AC-like state. RNA-velocity analysis of scRNA-seq data suggests that this may be true in many patient samples [8]. This in turn raises the important question of whether tumors with an increased proportion of AC-like cells may respond to injury with a more pronounced transition to a MES-like state. In support

of this idea, mouse tumors derived from NSCs as opposed to OPCs, which show an enrichment for AC-like signatures, are also enriched for MES-like transcripts [10,30]. However, an AC-like precursor state is unlikely to be essential because direct **proneural** to mesenchymal transition has been observed in several studies, particularly following treatment [81]. It would be important to ascertain in future studies whether astrocytic competency is required for the expression of a MES-like state in treatment-naive tumors.

### Regenerative processes are echoed in glioblastoma

In addition to restricting the area of injury and clearing harmful cellular debris, a final aspect of the normal injury response is to promote wound healing and regeneration. Although the ability of the healthy adult human brain to generate new neurons is a matter of ongoing debate [82], in the context of injury an increase in neurogenesis has been observed within the SVZ following ischemic stroke [83,84], and cells expressing markers of newborn neurons [doublecortin (DCX)<sup>+</sup>, polysialylated neuronal cell adhesion molecule (PSA-NCAM)<sup>+</sup>, and SRY (sex determining region Y)-box 2 (SOX2)<sup>+</sup>] are present at the infarct region of patients who have experienced either stroke [85] or TBI [86]. However, even if new neurons are generated, data from mice demonstrate that they largely fail to achieve long-term replacement, and eventually succumb to cell death [87]. Similar mechanisms are observed in glioblastoma, whereby NSCs migrate towards the tumor mass, a mechanism which is currently being explored as a means to deliver therapeutics through implantation of exogenous functionalized NSCs [88,89].

Glioblastoma cells, in line with their stemness properties, retain the potential to differentiate towards an immature neuronal-like state [90,91], as captured within the NPC-like subgroup which encompasses cells expressing degrees of stemness and differentiation within the neuronal lineage [7,8,23,90,91]. However, specific niche factors associated with NPC-like differentiation and the degree to which this phenotype recapitulates normal neurodevelopment versus regenerative signaling remain largely unexplored and would be an interesting topic for future investigation.

In contrast to neurogenesis, the reintegration of new oligodendrocytes is relatively straightforward and leads to more efficient cell replacement. Following acute insult, OPCs migrate to the site of injury and undergo a period of proliferation before differentiating and remyelinating axons [92,93]. Multiple signaling networks regulate these phases of oligodendrogenesis in response to injury and are intimately linked to the activity of microglia, leukocytes, and astrocytes via the release of cytokines, neurotrophins, and growth factors including TNF- $\alpha$ , FGF, IGF1, BDNF, and PDGF which expand the OPC population, as well as of molecules such as activin-A that can promote differentiation [94,95]. However, in chronic conditions these repair processes are limited and often fail to generate myelinating oligodendrocytes. One reason for this is that chronic inflammation can have detrimental effects on differentiation. For example, although TNF- $\alpha$  promotes OPC proliferation via TNF receptor type II (TNFR2) [96], cytokines TNF- $\alpha$  and IFN- $\gamma$  have both been shown to inhibit differentiation [97,98].

This process appears to be conserved in glioblastoma in that malignant cells have been found to differentiate in response to tumor-induced injury to white matter regions, thus acquiring an immature oligodendrocyte-like state [13,92,93] (Figure 1). Although tumor cells do not mature to form myelinating oligodendrocytes, as is also frequently the case for non-malignant oligodendrocytes in chronic injury as described above, this partial differentiation is sufficient to suppress proliferation and cell motility [13,14]. The extracellular signals that induce malignant cell differentiation remain to be determined, but an association between activated microglia and astrocytes was observed, as is found in normal injury-related oligodendrogenesis [94,99]. Interestingly, this phenotype was only present in tumors that contained a subset of cells with retained expression of SOX10, a key oligodendrocyte-lineage marker and master regulator [13,14]. This indicates

that only tumors capable of enacting oligodendrocyte-lineage programs undergo this regenerative-like response to injured white matter. Given that the frequency of OPC-like cells within glioblastoma is influenced by the cell-of-origin, as discussed above, one might hypothesize that cells derived from the malignant transformation of OPCs may be more prone to white matter injury-induced oligodendrocyte-like differentiation than NSC-derived tumors, and that this bias could be exploited for patient stratification and treatment [7,10,25].

A final and particularly pertinent aspect of the regenerative process for glioblastoma is the role of growth factors at the injury site that are likely to stimulate expansion and self-renewal of the stem-like/progenitor pool. TAMs and reactive astrocytes secrete potent mitogens including FGF and EGF ligands which are essential for the proliferation and self-renewal of glioma stem-like cells (GSCs) [100], as well as PDGF, a known driver of gliomagenesis [35,38,75,81,101,102]. Consistent with this, the density of TAMs expressing markers associated with an anti-inflammatory state correlates with tumor proliferation [103], and suppression of the microglial anti-inflammatory program can slow tumor growth in a proneural mouse model of glioblastoma [101]. Furthermore, suppressing microglia-driven astrocyte reactivity via janus kinase (JAK)/STAT inhibition in human *ex vivo* brain slices can also reduce the proliferation of subsequently implanted GSCs [68]. In addition to driving proliferation, EGF and FGF are sufficient to induce **dedifferentiation** of AC- and OPC-like differentiated tumor cells *in vitro* and *in vivo* [13,104], which could indicate that regenerative cues at injury sites shift tumor cells to a more stem-like state (Figure 2). Although this hypothesis remains to be tested *in vivo*, collectively this evidence suggests that signaling from reactive microglia and astrocytes, which evolved to aid in the regeneration of the nervous system, may be hijacked by malignant cells to fuel their growth and stemness.

### Therapeutic implications

The impact of injury on cell fate in glioblastoma has important therapeutic consequences because distinct subpopulations exhibit different sensitivities to treatment [4–6]. MES-like populations that form as a result of reactive injury-like programs exhibit enhanced resistance following irradiation [6,81,105]. It is possible that this resistance is in part related to their similarity to reactive astrocytes. The ability of astrocytes to respond to insults allows them to survive and to endure inflammation and oxidative stress. To this end, reactive astrocytes are resistant to Fas- and TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis [106] and exhibit enhanced radiation-induced DNA damage repair compared to non-reactive astroglial cells [107]. When transposed onto a malignant backdrop, these same reactive mechanisms may contribute to therapy resistance. Concordantly, MES-like tumors have been shown to exhibit an enhanced capacity for DNA repair following irradiation [6]. Future studies should examine whether the molecular underpinnings of enhanced DNA repair are common to both reactive astrocytes and MES-like tumor cells.

In addition, MES-like glioblastomas have been shown to express higher levels of the immune checkpoint molecule programmed cell death ligand 1 (PD-L1) compared to proneural tumors [108], and this may contribute to the highly immunosuppressive nature of this tumor subtype [76]. This property may again reflect the similarities between MES-like cells and reactive astrocytes. Reactive astrocytes upregulate PD-L1 as part of their neuroprotective response to injury, resulting in inhibition of T cell responses, and thereby protecting against excessive neuroimmune and neuroinflammatory responses and attenuating brain damage [109,110]. Because immune evasion is a major therapeutic hurdle, it stands to reason that approaches that suppress MES-like reactivity identified through increased understanding of injury/inflammatory pathways may improve treatment outcomes.

Stem-like cells, which lie at the apex of the neurodevelopmental hierarchy, have long been acknowledged to display enhanced resistance to chemo- and radiotherapy, and are believed



to be largely responsible for driving tumor recurrence [4,5,111]. It has been posited that this resistance may reflect the enhanced capacity for DNA repair observed in non-malignant stem cells which allows the perpetuation of intact genomes to their progeny [112]. Similarly, enhanced DNA repair mechanisms are observed in GSCs and are believed to contribute to treatment resistance [4,5]. It therefore stands to reason that injury signals that maintain stemness and self-renewal, such as EGF and FGF described above, would contribute to treatment resistance. Conversely, in injury microenvironments that promote prodifferentiation regenerative responses, such as infiltrated white matter, cells would be predicted to become less resistant. In support, stem-like-to-neuronal transition has been shown to reduce tumorigenicity and increase radiation sensitivity *in vivo* [91]. Although the relative sensitivity of the OPC-like population is currently unknown, normal oligodendrocytes are particularly vulnerable to oxidative stress. It is therefore tempting to speculate that NPC-like and OPC-like cells, which are encompassed by the proneural signature, may be more vulnerable to radiation than GSC or MES-like cells, and that this sensitivity may, in part, contribute to the proneural to mesenchymal transition observed following radiation therapy [6,113].

A final important consideration regarding the role of injury in glioblastoma is how treatment-induced injury may impact on tumor biology and contribute to recurrence. Surgical resection, radiotherapy, and chemotherapy all cause tissue damage and cell death [20–22,114] which, as described, may influence malignant cell fates in prognostically and therapeutically meaningful ways. The contribution of treatment-induced injury to glioma biology has thus far been largely neglected, but with new discoveries on injury responses in glioma, it is emerging as an important area of investigation. About 90% of tumors reoccur within a few centimeters of the resection margin [115]. The higher density of residual cancer cells in this region has often been proposed as the main underlying reason. However, some limited evidence suggests that injury processes within the post-treatment tumor margin could also be at play [21,101].

Owing to difficulties in accessing tissue within an acute and consistent post-treatment window in humans, most studies examining the immediate or early response of the margin microenvironment and residual tumor cells to treatment have been carried out in animal models. Such work has demonstrated that irradiation is associated with an acute accumulation of TAMs in the peritumoral area, which are not observed at later timepoints or in recurrent tumors [102]. Such accumulation may contribute to resistance because depleting TAMs or blocking infiltration of bone marrow-derived macrophages has been shown to synergize with radiation therapy to extend survival [101,102]. Irradiation can also induce normal astrocytes to become reactive or even senescent, further contributing to inflammation within the post-treatment microenvironment [116,117]. In addition to irradiation, surgical resection is also likely to profoundly alter the microenvironment at the tumor margin. Indeed, there is some evidence that resection may increase the density of reactive astrocytes in the peritumoral region, and this may enhance tumor cell proliferation and migration [21].

This acute, reactive post-treatment microenvironment would be predicted to promote a MES-like state. Indeed, analysis of proneural glioblastoma mouse models has shown an enrichment of injury-associated MES-like populations following irradiation [6,81,105]. In line with this, bulk genomic approaches comparing primary and recurrent human glioma samples reveal that around half to two-thirds of tumors switch subtype following treatment, and that the MES-like subtype is the most stable [33,118]. The variability in penetrance may, in part, be due to the variation in time to the second surgical intervention, as well as to regional sampling bias. Nonetheless, when early and late recurrences have been compared, the proportion of immune cells and reactive astrocytes has been found to be elevated in early recurrences, suggestive of the presence of an acute treatment-induced inflammatory process as observed in animal models [72].

## Concluding remarks and future perspectives

Injury in its plethora of forms is a major threat to the homeostasis of the brain, and hence robust biological processes have evolved to minimize and repair tissue damage. As described in this review, injury is inextricably linked to tumor expansion. Biological processes designed to protect the brain are preserved in malignancy and shape cellular phenotypes through the acquisition of reactive astrocyte features, expansion of progenitor-like cells, or differentiation towards an oligodendrocyte fate. Although many knowledge gaps remain (see [Outstanding questions](#)), it is anticipated that further injury-driven phenotypes will be uncovered in the context of glioblastoma, and that these phenotypes may be regulated by various spatial and temporal cues. Considering the observed differences in tumorigenicity and resistance between cell states, blocking mesenchymal transition or promoting injury-induced differentiation may provide new ways to control tumor growth and enhance treatment sensitivity. A crucial consideration is the impact of injury microenvironments on residual margin cells following treatment. Although the pronounced molecular heterogeneity of glioblastoma hampers targeted therapies, collapsing reactive injury programs may prove to be a more successful strategy for blocking or slowing down recurrence. More generally, determining the contribution of injury to glioblastoma progression and recurrence has the potential to uncover more effective treatments.

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## Declaration of interests

The authors declare no conflicts of interests.

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## Outstanding questions

The growing tumor mass in glioblastoma has been shown to physically damage the surrounding brain tissue. What are the precise mechanisms by which the growing tumor injures the brain?

What is the specific role of neuronal injury (including cell-autonomous processes, axon-specific pathways, and Wallerian degeneration) in the behavior of glioblastoma, and can neuroprotective strategies reduce or alter the course and manifestations of disease?

Can we identify the injury-related microenvironmental differentiation factors that are sufficiently potent to overcome oncogenic signaling and exploit them to suppress tumor growth?

To what degree is tumor proliferation dependent on injury-related neurotrophic/growth factors that maintain stemness, and is this specific to the tumor bulk?

Is there a niche for neural progenitor cell-like differentiation? If so, does it correlate with injury microenvironments, by analogy to the well-known increase in the production of normal neural progenitor cells in response to brain injury?

Standard-of-care treatments in glioblastoma cause tissue damage and cell death. Do injury responses at the tumor resection margin contribute to tumor recurrence, and, if so, how?

Would targeting injury programs that appear to be conserved across patients prevent recurrence, independently of genetic heterogeneity?

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