BRAIN
SCIENTIFIC COMMENTARY

Forced but effective partners in crime: how astrocytes drive the progression of glioblastoma

This scientific commentary refers to 'Astrocyte immunometabolic regulation of the tumour microenvironment drives glioblastoma pathogenicity' by Perelroizen *et al.* (https://doi.org/10.1093/brain/awac222).

Glioblastoma multiforme (GBM) is the most common and malignant glioma in adults. Patients diagnosed with GBM face the dismal prognosis of a median life expectancy of ~15 months. As the tumours progress, patients are likely to experience severe complications, such as epileptic seizures, peritumoural oedema, and intracranial hypertension. The extreme aggressiveness of GBM is the result of several pathological features. For instance, GBM spreads diffusely throughout the CNS, enabling it to escape surgical interventions. When GBM pushes its way through the parenchyma, it causes severe tissue damage and disrupts CNS functions. Moreover, GBM is effective at withstanding conventional radiation and chemotherapies.[1]

One major factor underlying the malignant properties of GBM tumours is their ability to use the cellular CNS microenvironment to their advantage. Among the cells within this microenvironment are microglia, the resident CNS macrophages, which generally act as a 'rapid response force' to emerging pathological conditions. When GBM compromises blood–brain barrier integrity, peripheral macrophages cross over into the CNS and colonize the peritumoural regions. Microglia and peripheral macrophages can constitute up to 40% of the tumour cell mass, and are actively attracted to GBM via chemokines (Fig. 1). However, GBM prevents these 'roped in' microglia and macrophages from acquiring tumour-opposing phenotypes. Instead, microglia and macrophages are quickly turned into accessories, which promote GBM cell proliferation, invasive growth, and therapy resistance. Indeed, tumour-associated microglia and macrophages can suppress immune cells and thus interfere with anti-cancer immunotherapies.[2] However, it is unknown whether the tumour-promoting and immunosuppressive properties of microglia and macrophages are induced directly by GBM or indirectly via non-neoplastic cells in the tumour micro-environment.

Besides microglia and macrophages, GBM also exploits astrocytes—the most abundant CNS glial cells. Astrocytes regulate multiple processes in the CNS; they maintain essentially all forms of homeostasis, provide metabolic and trophic support, modulate neurovascular coupling, and regulate synaptogenesis, as well as synaptic transmission and plasticity. In addition, astrocytes activate a defence mechanism known as reactive astrogliosis to protect the vulnerable CNS from pathological conditions. Astrocyte reactivity is accompanied by substantial changes in astrocyte function and morphology, such as the coordinated outgrowth of processes to form damage-containing scars. When astrocytes encounter gliomas, they attempt to create isolating scars around the tumours (Fig. 1). However, in GBM, astrocytes are unable to contain the neoplastic cells. On the contrary, GBM turns the astrocytes into enhancers of tumour progression. Studies have shown that GBM-associated astrocytes release tumour-promoting molecules such as trophic factors, matrix metalloproteinases, and microRNAs.[3] However, it has been unclear so far to what extent astrocytes contribute to GBM pathogenicity.

In this issue of *Brain*, Perelroizen and co-workers[4] reveal key roles of astrocytes in GBM progression using a multidisciplinary approach. First, the authors tackle GBM-associated astrocytes directly using genetic ablation techniques. For this purpose, they exploit the hallmarks of reactive astrogliosis inherent in tumour-associated astrocytes. Analogous to reactive astrocytes in other neuropathological conditions, tumour-associated astrocytes express high levels of glial fibrillary acidic protein (GFAP) and are proliferative. In their current study, Perelroizen *et al.* used two mouse models that allow inducible ablation of tumour-associated astrocytes: GfapCre:iDTR mice, in which astrocytes with high GFAP levels can be ablated, and Gfap-TK mice, in which proliferative astrocytes can be targeted. Eliminating tumour-associated astrocytes in GL261 glioma xenografts borne by GfapCre:iDTR (GL261-GfapCre:iDTR) and Gfap-TK (GL261-Gfap-TK) mice halted tumour progression in both models. GL261-Gfap-TK mice, however, were better suited for long-term studies since fatal bowel inflammation occurred in GL261-GfapCre:iDTR animals as a consequence of ablating GFAP+ enteric glia. Nevertheless, the GL261-Gfap-TK model showed that depleting tumour-associated astrocytes not only stalls GBM progression but drives the tumours into regression and prolongs animal survival.

Pereloizen *et al.* also performed in-depth analyses to dissect changes in the transcriptomes of glioma-associated astrocytes. They found that transcript patterns indicative of tumour-associated astrocytes may serve as direct inducers of microglia and macrophage chemoattraction and immunosuppression. These results are consistent with previous data from *ex vivo* assays and GBM patients.[5,6] The authors used elaborate combinations of cell culture and *in vivo* experiments to support their transcriptome-based findings. For instance, they showed that media from GL261 glioma triggered in separately cultivated astrocytes the secretion of macrophage-attracting factors. These astrocytic factors can

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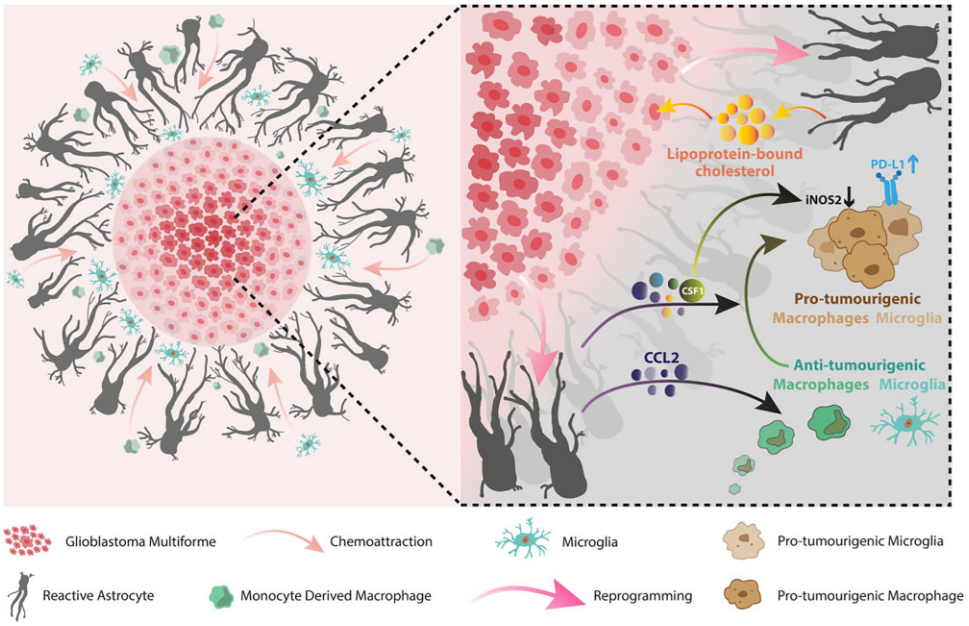

Figure 1 Astrocyte-dependent processes in the micro-environment of glioblastoma multiforme. *Overview*: GBM tumours are encircled by astrocytes, which initially attempt to form isolating scars. The GBM also draws microglia and monocyte-derived macrophages into peritumoural areas. *Close-up*: GBM reprogrammes astrocytes into a tumour-promoting state. Conditioned astrocytes then secrete factors such as CCL2, which attracts microglia and macrophages into the tumour mass. Astrocyte-derived factors prevent microglia and macrophages from acquiring tumour-opposing phenotypes, e.g. via the CSF1-dependent downregulation of microglial inducible nitric oxide synthase (iNOS). GBM-associated astrocytes induce the expression of tumour-promoting genes in microglia and macrophages, including immunosuppressive factors, such as PD-L1. Tumour-associated astrocytes provide essential metabolic support to GBM via lipoprotein-bound cholesterol.

induce the directed migration of isolated monocytes. Furthermore, by using neutralizing antibodies, Perelroizen *et al.* pinpointed CCL2 as the major macrophage chemoattractant generated by GBM-reprogrammed astrocytes (Fig. 1). The *in vitro* monocyte migration assays correlate well with the team's GL261-Gfap-TK mouse model, in which the ablation of peritumoural astrocytes also inhibits the recruitment of peripheral macrophages to GBM tumours.

Further transcriptomics analyses revealed that peritumoural astrocyte ablation also downregulates the expression of key tumour-promoting factors in glioma-associated microglia and macrophages. These include programmed cell death ligand 1 (PD-L1/CD274), a transmembrane protein that inhibits the proliferation and cytotoxic activities of immune cells and that is implicated in the failure of anti-cancer immunotherapies (Fig. 1).[7] In cell culture and *in vivo* experiments, microglia significantly lower their PD-L1 levels once the tumour-associated astrocyte influence is removed. Besides chemoattraction and immunosuppression, GBM also uses astrocytes to prevent microglia from becoming biochemical hazards: pro-inflammatory microglia possess the means to kill neighbouring cells by releasing nitric oxide (NO).[8] Tumour-associated astrocytes avoid NO-mediated glioma toxicity by forcing microglia to downregulate inducible nitric oxide synthase (iNOS). Perelroizen *et al.* identify astrocyte-derived CSF1 as an inhibitory signal upstream of microglial iNOS (Fig. 1). In summary, tumour-associated astrocytes play a key role in shaping macrophages and microglia into tumour-promoting cells.

In addition, Perelroizen *et al.* investigated the metabolic functions of tumour-associated astrocytes. The team focused on cholesterol, which may be a limiting factor for GBM because these cancer cells must grow in an organ that is almost completely isolated from the supply of cholesterol from the liver. Local *de novo* cholesterol synthesis is thus an integral process in CNS metabolism. Several therapeutic strategies in development aim to interfere directly with cholesterol metabolism in GBM cells.[9] Perelroizen and colleagues, on the other hand, suggest an alternative strategy involving astrocytes in the tumour microenvironment. In cell culture experiments, the authors show that statins inhibit cholesterol synthesis, causing significant cell death of non-transformed astrocytes but not of GBM cells. In contrast, GBM are sensitive to removal of extracellular lipoprotein-bound cholesterol, whereas this has no effect on the viability of astrocytes. These results indicate that cholesterol secreted by astrocytes covers the metabolic needs of GBM (Fig. 1). Furthermore, Perelroizen *et al.* show that GBM-dependent reprogramming increases the expression of cholesterol-synthesizing enzymes as well as the cholesterol efflux transporter ABCA1 in astrocytes. ABCA1, one of 13 ABC transporters expressed in the CNS, is essential for the astrocyte-dependent cholesterol supply of GBM. ABCA1 knockdown in tumour-associated astrocytes in GL261 xenograft-bearing mice significantly reduced cholesterol stores in GBM cells. Moreover, interfering with this cholesterol efflux route also resulted in glioma regression and prolonged animal survival.

In summary, Perelroizen *et al.* show convincingly that GBM requires astrocytes for metabolic support and to reprogramme microglia/macrophages in order to shape a tumour promoting microenvironment. Astrocytes and/or astrocyte-dependent processes may therefore provide new opportunities to target GBM, where approaches centred on cancer cells have so far failed. To identify potential therapeutic targets, further research is needed to uncover the molecular mechanisms underlying the reprogramming and tumour-promoting functions of GBM-associated astrocytes.

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Competing interests

The authors report no competing interests.

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