Glioblastomas are hierarchically organised tumours driven by glioma stem cells that retain partial differentiation potential. Glioma stem cells are maintained in specialised micro-environments, but whether, or how, they undergo lineage progression outside of these niches remains unclear. Here we identify the white matter as a differentiative niche for glioblastomas with oligodendrocyte lineage competency. Tumour cells in contact with white matter acquire pre-oligodendrocyte fate, resulting in decreased proliferation and invasion. Differentiation is a response to white matter injury, which is caused by tumour infiltration itself in a tumour-suppressive feedback loop. Mechanistically, tumour cell differentiation is driven by selective white matter upregulation of SOX10, a master regulator of normal oligodendrogenesis. SOX10 overexpression or treatment with myelination-promoting agents that upregulate endogenous SOX10, mimic this response, leading to niche-independent pre-oligodendrocyte differentiation and tumour suppression in vivo. Thus, glioblastoma recapitulates an injury response and exploiting this latent programme may offer treatment opportunities for a subset of patients.
glioblastoma (GBM) is the most common and malignant primary brain tumour. Stark resistance to current treatments, which include maximal surgical resection, chemoradiotherapy, leads to tumour relapse in virtually all patients with a median survival of less than 15 months. GBM initiation, growth and recurrence are thought to be rooted within a subpopulation of therapy-resistant tumour cells with properties of normal neural stem cells, termed glioma stem cells (GSCs).

Mounting evidence indicates that GSCs fuel tumourigenesis by recapitulating normal neural lineage hierarchies of quiescence, self-renewal and generation of non-dividing progeny. Fate decisions of normal neural stem cells are tightly controlled by the microenvironment, which maintains stemness in specialised niches and directs differentiation along the appropriate lineages. Similarly, it is well established that GSCs are maintained in perivascular and hypoxic regions of the tumour bulk. In contrast, little is known about the differentiation potential of GSCs within tumours and whether pro-differentiative niches exist. However, improved understanding of lineage progression central to our understanding of the disease and may reveal novel strategies to suppress GBM growth and recurrence by directing GSC fate towards a mature, non-dividing state.

Consistent with this idea, manipulation of candidate developmental pathways to promote tumour cell differentiation into astrocyte and neuronal lineages has shown efficacy in preclinical models.

Diffuse infiltration into the normal brain parenchyma is a hallmark of GBM and underlies recurrence by precluding complete surgical resection. As GBM cells infiltrate away from the tumour bulk, they are confronted with new and heterogeneous microenvironments, which would be predicted to affect fate decisions. Indeed, it has been proposed that invading GSCs may lose stemness, which remains controversial, with studies both in support and against this idea. A predominant route of infiltration is the white matter, which consists of bundles of myelinated axons, known as tracts, and represents approximately 2–5% of brain tissue, and the striatum (ST) as a region of grey matter, were extending into the corpus callosum (CC) as a region of white matter, and the striatum (ST) as a region of grey matter, were transitioning.

Tumour cells were only found inside the tumour bulk. The observed differentiation block of GBM cells is likely due to amplification of PDGFRA in this line, a key negative regulator of developmental oligodendrocyte differentiation which is commonly mutated in proneural/OPC-like GBM cells. Indeed, overexpression of constitutively active PDGFRA in cultured mouse neural stem cells arrested their differentiation at the O4+ pre-oligodendrocyte stage, confirming that failure to switch off PDGFRA signalling plays an important role in preventing terminal differentiation of tumour cells (Supplementary Fig. 1n–p). Thus, GBM cells differentiate to a pre-oligodendrocyte/immature pre-oligodendrocyte state in white matter, which is marked by induction of SOX10 expression.

To determine the generality of these findings, we examined SOX10 expression in the TCGA dataset and in a panel of primary GSC lines by qPCR. Consistent with previous studies, we found micro-dissected under fluorescence guidance (Fig. 1a). Tumour tissue was dissociated to single cells and GFP+ tumour cells FACS-purified, pooled and processed for RNA-sequencing (RNA-seq). Bioinformatics analysis of human tumour reads revealed that margin cells are transcriptionally distinct from bulk cells, with invasive cells acquiring markers of lineage progression towards astrocytes, as well as downregulating proliferation signatures, which resulted in decreased EdU incorporation in vivo (Fig. 1b, Supplementary Fig. 1a–e, Supplementary Table 1 and Supplementary data 1–3).

Strikingly, it also indicated that the transcriptomes of CC and ST tumour cells differed from one another (Fig. 1b). Tumour cells invading into white matter selectively upregulated signatures of oligodendroglia, suggesting that myelinated regions may promote progression along the oligodendrocyte lineage (Fig. 1c, Supplementary Fig. 1f and Supplementary Table 1, and Supplementary data 1–3). In agreement with this, CC cells upregulated a panel of oligodendrocyte lineage marker genes, including the transcription factor SOX10, a master regulator of oligodendrogenesis (Fig. 1d). Notably, upregulation of myelin genes was mild and incomplete in the CC, indicative of partial differentiation, as expected from cancer cells. To determine whether these gene expression changes also correlated with phenotypic changes, we characterised the differentiation response at the single cell level in two sets of preclinical experiments. First, we dissociated G144 cells from the CC, ST and B of xenografts, seeded them acutely in mitogen-free media and assessed expression of SOX10, the pre-oligodendrocyte marker O4 and the myelinating oligodendrocyte marker myelin basic protein (MBP) by immunocytochemistry (Supplementary Fig. 1g, h). Preparations isolated from the CC had a strong increase in the proportion of cells positive for SOX10, the majority of which also expressed O4, but not MBP, confirming that transcriptomic signatures reflect changes in tumour cell fate.

Second, we examined lineage marker expression within xenografts in situ by immunohistochemistry. GFP or the human-specific nuclear antigen (HuNu) were used to label tumour cells and distinguish them from endogenous mouse glia (Supplementary Fig. 1i, j). The majority of tumour cells began to express high levels of SOX10 in white matter, including in myelinated fibres inside the tumour bulk (Fig. 1e, f). SOX10+ tumour cells were significantly less proliferative than tumour cells that remained SOX10− within the same region (Fig. 1g, h) and occasionally expressed the immature oligodendrocyte markers CNP and CC1 (Supplementary Fig. 1k, l), but were again negative for the mature marker MBP (Supplementary Fig. 1m). To further assess the white matter specificity of this response, we implanted G144 cells directly into cortical grey matter or in the corpus callosum and examined their differentiation using SOX10 induction as a readout. Differentiated SOX10+/EdU− tumour cells were only found in the CC, confirming that the white matter selectively promotes partial GBM differentiation (Fig. 1i,j).

The observed differentiation block of GBM cells is likely due to amplification of PDGFRA in this line, a key negative regulator of developmental oligodendrocyte differentiation which is commonly mutated in proneural/OPC-like GBM cells. Indeed, overexpression of constitutively active PDGFRA in cultured mouse neural stem cells arrested their differentiation at the O4+ pre-oligodendrocyte stage, confirming that failure to switch off PDGFRA signalling plays an important role in preventing terminal differentiation of tumour cells (Supplementary Fig. 1n–p). Thus, GBM cells differentiate to a pre-oligodendrocyte/immature pre-oligodendrocyte state in white matter, which is marked by induction of SOX10 expression.

Results

Region-specific GBM transcriptomes. Despite the fundamental role of white matter in GBM biology, how this specialised microenvironment might modulate tumour cell behaviour remains unknown. To probe white matter phenotypes, we made use of a well characterised patient-derived GSC line with propensity to invade along white matter tracts (G144). GFP-labelled G144 cells were stereotactically injected into the striatum of immunocompromised mice and, upon development of clinically apparent disease, the tumour bulk (B) and margin regions extending into the corpus callosum (CC) as a region of white matter, and the striatum (ST) as a region of grey matter, were
that a subset of patient tumours (~18%) expressed high levels of SOX10. SOX10 high tumours were enriched for the proneural, but also included mesenchymal and classical transcriptional subtypes, as well as IDH1 mutant tumours, suggesting that pre-oligodendrocyte differentiation may occur across all GBMs (Supplementary Fig. 2a, b)\(^9\). In addition, a subset of primary GSC lines retained above average SOX10 expression in vitro, confirming cell-intrinsic expression in tumour cells (Supplementary Fig. 2c and Supplementary Table 2). We next xenotransplanted five SOX10+ GSC lines (three of which were freshly dissociated from primary tumours) and examined their behaviour in white matter. One line had lost white matter regulation of SOX10 induction, displaying homogeneous high SOX10 expression throughout the xenograft and was not analysed further. In contrast, the other four lines were similar to G144 cells, with a somewhat variable, but consistent increase in the proportion of SOX10+ cells in white matter as compared to grey matter (Fig. 2a, b and Supplementary Fig. 2d). In addition, as in G144 xenografts, SOX10+ cells proliferated significantly less than SOX10- cells in white matter and partially progressed to CNP+ immature pre-oligodendrocyte cells (Fig. 2c and Supplementary Fig. 1k, l and Fig. 2e). To determine whether the white matter-induced response also occurs in primary patient tumours, we took two complementary approaches. First, we selected three SOX10+
cases based on retained SOX10 expression in derivative GSC lines (GCGR-L12, GL67 and GL23). We found that in all cases the white matter contained SOX2+ tumour cells, which upregulated SOX10 and proliferated less than their SOX10− counterparts, confirming that GSC behaviour in xenograph models reflects disease phenotypes (Fig. 2d, e and Supplementary Table 3). SOX2 staining was specific to GBM cells, as the vast majority of endogenous SOX10+ OPGs were negative for SOX2 in areas of tumour infiltration (Supplementary Fig. 3a). Second, we selected 23 white matter-containing cases without prior knowledge of SOX10 status. Remarkably, we found that 15 cases contained at least a subset of SOX10+ cells in white matter, regardless of molecular characteristics (Fig. 2f, Supplementary 3b and Supplementary Table 3). In addition, SOX10− cells were largely proliferative, progressed to a CNP+/CC1+/MBP− immature pre-oligodendrocyte state and were restricted to MBP+ tumour regions (Fig. 2e and Supplementary Fig. 3c–e). Finally, in a somatic Nfi1/Pten/p53 CRISPR-based mouse model, while the vast majority of tumour cells were SOX10−, a much greater proportion of tdTomato+ tumour cells differentiated to CC1+/CNP+/Ki67− immature oligodendrocyte cells in the white matter relative to the grey matter (Supplementary Fig. 3f–j). These experiments confirm that a subset of GBMs progress to pre-oligodendrocyte-like cells in white-matter.

Disrupted white matter drives pre-oligodendrocyte differentiation. Next, we sought to understand what properties of white matter are differentiation-promoting. We noticed that SOX10 induction was most robust in areas where OS expression appeared disrupted, suggesting that it might be linked to demyelination (Supplementary Fig. 1i). We therefore assessed myelin integrity in xenograph by fluoromyelin staining and by electron microscopy (EM). The proportion of SOX10+/HuNu+ tumour cells was highest in heavily infiltrated, fluoromyelin negative, white matter areas, as compared to dye-positive, low tumour-density, myelin regions in four independent xenograph cases (Fig. 3a, b and Supplementary Fig. 4a). A similar differentiation pattern was also found in patient tissue, where only few SOX2+ tumour cells upregulated SOX10 in intact myelin (Supplementary Fig. 4b). EM analysis of G144 xenographs further revealed severe axonal pathology and demyelination in highly infiltrated white matter regions, including axonal swelling and vacuolisation, presence of dark axons, myelin decompaction and a significant increase in g-ratios relative to contralateral normal brain (Fig. 3c–i and Supplementary Fig. 4c). In contrast, g-ratios remained normal in the tumour-infiltrated striatal grey matter (Fig. 3h), indicating that demyelination is specific to white matter. Consistent with this, activated microglia increased selectively in tumour-infiltrated white matter and endogenous oligodendrocytes were significantly decreased in the CC (Supplementary Fig. 4d–g). Furthermore, activated microglia with engulfed myelin debris was frequently observed in both xenographs (Supplementary Movie File 1) and human samples, and colocalised with SOX10+ tumour cells (Supplementary Fig. 4h–j). Surprisingly,
despite severe myelin dysfunction, the mice did not exhibit overt motor or cognitive deficits. Super resolution and correlative light and electron microscopy (CLEM) showed that within areas of myelin disruption tumour cells associated closely with both intact myelinated and demyelinating axons (Fig. 3j, k). This did not affect tumour cell survival (Supplementary Fig. 4k, l), but occasionally resulted in tumour cells acquiring oligodendrocyte morphology, in line with a differentiation response (Supplementary Fig. 4m).

Together, this data supports a model whereby tumour infiltration into the white matter induces an injury-like microenvironment, which in turn triggers GBM differentiation in a tumoursuppressive feedback loop. To test this more directly, we carried out a time-course analysis of the response of GBM cells...
Fig. 3 Exposure to disrupted myelin drives GBM progression down the oligodendrocyte lineage. a SOX10 (grey), fluoromyelin (FM, red), DAPI (blue) immunofluorescence of GFP+ G144 xenografts (PD144). Arrowheads denote disrupted areas. Scale = 500 μm. b % SOX10+ tumour cell in areas of high (blue)/low (grey) disruption in indicated xenografts. ≥120 cells across ≥6 ROIs per region. n = 4 xenografts. p = 0.03, Mean, paired one-tailed Student’s t test. c Electron micrographs of myelin bundles in the ipsilateral (infiltrated) and contralateral (intact) striatum of PD144. Scale = 1 μm. d–i, quantification of indicated axonal and myelin phenotypes in EM data from b. n = 3–4 xenografts. d p = 0.03, f, p = 0.03, g p < 0.0001, h p = 0.01, i p = 0.01, **p < 0.01, *p < 0.05. Mean ± SEM, paired one-tailed Student’s t test, 2-way ANOVA or linear regression. j % GFP+ tumour cells in contact with axons. ≥300 SOX10+/SOX10− cells across 3 ROIs. Mean ± SEM, n = 3 tumours, p < 0.0001. Unpaired two-tailed Student’s t test. k Correlative light and electron micrograph (CLEM) of tumour cell directly interacting with multiple white matter axons. ii–iv magnifications of i highlighting interactions with decompacted myelin (A, B), naked (C), and intact myelinated axon (D) Scale = 10 μm (i), 5 μm (ii), 1 μm (iii, iv). l–s time-course analysis of tumour cell differentiation and gli response during corpus callosum (CC) infiltration in PD144. l quantification of FM intensity relative to tumour density. Dots indicate individual xenografts colour-coded by time-point. n = 3 tumours/time point, n = 2 control brains. R2 = coefficient of determination. m–s quantification of indicated cell types over time. Minimum ROI = 300 μm. Mean ± SEM, n = 3 xenografts. m 10wk p = 0.005, 12wk p = 0.0009, n 8wk p = 0.02, 10wk p = 0.02, 12wk p = 0.0005, o 4wk p = 0.0009, 6wk p = 0.0003, 8wk p = 0.0002, 10wk p < 0.0001 12wk p < 0.0001, r 6wk p = 0.0003, 8wk p < 0.0001, 10wk p < 0.0001, 12wk p < 0.0001, s 10wk p = 0.01, 12wk p = 0.0014, one-way ANOVA with Dunnett’s multiple comparison tests. t SOX10 (red), MBP (grey), EdU (turquoise), DAPI (blue) immunofluorescence of GFP+ G144 cells directly injected (injured CTX) or invaded into the inner cortex from the tumour bulk (invaded CTX). Dotted lines delineate the corpus callosum (CC). Scale = 100 μm. u % SOX10+/EdU− differentiated tumour cells shown in t ≥340 cells per xenograft. Mean ± SEM, n = 3 xenografts per group. p = 0.01. Unpaired two-tailed Student’s t test.

Fig. 4 Differentiation depends on continuous exposure to white matter. a schematic representation of experimental workflow. b Kaplan Meier survival plot of nude mice injected with G144 cells acutely isolated from the bulk, corpus callosum (CC) and striatum of primary xenografts. n = 6 mice/group. c Representative images of secondary GFP+ tumours stained for neurofilament (grey) to identify axonal bundles, SOX10 (red) and DAPI (blue). Tumour areas from the indicated brain regions are shown revealing a differentiation pattern identical to primary lesions. Scale = 50 μm.

and the CC microenvironment to tumour infiltration. G144 xenografts were collected at 2 weeks intervals from full engraftment at 4 weeks to symptomatic disease at 12 weeks post-implantation and subjected to immunohistochemistry for differentiation and activated glia markers. Tumour infiltration resulted in progressive loss of myelin integrity, which correlated with a gradual increase in astrocite reactivity, microglia activation, OPC activation and oligodendrocyte death, all hallmarks of the glial response to brain injury (Fig. 3l–r, Supplementary Fig. 5a–d). Importantly, tumour cell differentiation paralleled these changes, with the number of SOX10+ /EdU− tumour cells gradually increasing over time and remaining low proliferative, indicative of stable pre-oligodendrocyte differentiation (Fig. 3s, Supplementary Fig. 5e).

To determine whether injury is causal to tumour differentiation, we next performed gain–of-function experiments. G144 cells were injected directly into the densely myelinated inner layers of the cortex, a brain region in which tumour cells frequently infiltrate from the striatal tumour bulk, but do not differentiate (Fig. 3t). Remarkably, the stab-wound injury caused by the needle (evidenced by induction of astrocyte reactivity and microglia activation, Supplementary Fig. 5f–h) was sufficient to induce GBM cell differentiation to SOX10+ /EdU− cells (Fig. 3t, u). Thus, GBM differentiation is a white matter injury-like response.

Differentiation is niche-dependent. To determine the stability of white-matter induced differentiation, we carried out secondary xenografts. G144 cells were isolated from CC, ST and B xenograft regions as above, and immediately re-injected in the striatum of secondary hosts (Fig. 4a). Survival analysis indicated that all cells, regardless of region of origin, formed tumours with similar latency, indicative of comparable tumourigenic potential (Fig. 4b). Furthermore, independent of initial SOX10 levels, all secondary lesions recapitulated SOX10 expression patterns of the primary tumours, which was high in white matter and low in grey matter regions and bulk (Fig. 4c). This suggests that once removed from white matter, pre-oligodendrocyte tumour cells may de-differentiate back to a GSC state. To directly test this hypothesis, we used the surface marker O4 together with GFP to FACS-purify pre-oligodendrocyte tumour cells from myelin-rich CC and B regions of xenografts, seeded them acutely in vitro in the presence of mitogens and monitored their morphology and proliferation in real-time by time-lapse microscopy (Supplementary Fig. 6a, b). Consistent with the in vivo results, we found that approximately 20% of branched, pre-oligodendrocyte O4+ tumour cells retracted their processes, re-acquired GSC morphology, and re-entered the cell-cycle (Supplementary Fig. 6b and Supplementary Movie Files 2, 3). Thus, maintenance of pre-oligodendrocyte fate in vivo requires continuous exposure to white matter.
SOX10 drives differentiation. In normal oligodendrogenesis, SOX10 is essential for lineage progression, differentiation and myelination\(^{32-34}\). We therefore asked whether the increase in SOX10 observed in white matter may also play a causal role in the induction of tumour cell differentiation. To address this, we transduced G144 cells with an inducible Tet-ON SOX10 overexpression construct and profiled vehicle- and doxycycline (Dox)-treated cultures by RNA-seq at 48 h post-induction. This time point was chosen to maximise the specificity of detected transcriptional changes and enrich for direct SOX10 targets. Immunofluorescence analysis confirmed detectable SOX10 protein expression in approximately 60% of the cells (Supplementary Fig. 6c, d). We identified 186 differentially expressed genes, the majority of which were upregulated (157, Supplementary data 4). Approximately 40% of SOX10-induced genes overlapped with genes upregulated in the corpus callosum in vivo, alongside significant overlap with signatures of oligodendrocyte lineage cells (Fig. 5a). These genes included both immature and mature oligodendrocyte markers, such as GPR17, ERBB3 and the myelin genes PLP1, CLA111, MYRF and UGT1 (Fig. 5b). Furthermore, GO analysis identified enrichment for terms associated with oligodendrocyte differentiation, including axon ensheathment, myelination, cell adhesion and positive regulation of gliogenesis (Fig. 5c and Supplementary data 5). Thus, SOX10 controls a transcriptional programme of oligodendrogenesis in tumour cells.

To functionally assess effects of this programme on tumour cell phenotypes, we overexpressed SOX10 in a panel of patient-derived GSC lines. This included two lines with ability to spontaneously differentiate to pre-oligodendrocytes (G7, G144). Cells were transduced with lentiviral vectors expressing constitutive SOX10 (SOX10 OE), or control empty vectors, and subjected to differentiation by growth factor withdrawal for 7 days\(^{35}\). SOX10 overexpression induced 7 of the 12 lines to generate O4\(^+\) pre-oligodendrocytes, regardless of basal differentiation competency, and, remarkably, decreased proliferation in all lines, as measured by Edu incorporation (Fig. 5d–g). This indicates that SOX10 is sufficient for GMB cell differentiation. To determine if it is also necessary, we knocked-out SOX10 by gene editing in G144 cells and carried out differentiation assays as above. We found that differentiation to O4\(^+\) cells was fully abolished in the absence of SOX10 (Fig. 5h–j). Together, these experiments demonstrate that SOX10 functions as a master regulator of pre-oligodendrocyte fate in GMB and confirm that corpus callosum phenotypes in vivo are largely mediated by SOX10 upregulation.

Increased SOX10 suppresses tumourigenesis. Our results so far suggest that manipulations capable of increasing SOX10 levels niche-independently should suppress tumourigenesis by promoting stable differentiation. To test this more directly, we performed intracranial transplantsations of luciferase and GFP-tagged G144 cells transduced with SOX10 OE or Control lentiviruses and monitored tumour growth and disease-free survival. SOX10 overexpression significantly delayed tumourigenesis, resulting in an overall increase in median survival from 65 to 91 days (Fig. 6a, b). To understand the mechanisms responsible, we examined Control and SOX10 OE tumours at 4 weeks post implantation by immunofluorescence analysis. Edu labelling revealed a marked decrease in the total number of proliferating tumour cells in SOX10 OE compared to Control lesions (Fig. 6c, d). Importantly, time-course analysis of SOX10 levels in the tumours that eventually formed revealed that SOX10 overexpressing cells were progressively outcompeted by cells that had escaped transduction, confirming that high SOX10 levels are tumoursuppressive (Supplementary Fig. 6e).

Although SOX10-mediated differentiation was most pronounced in invasive tumour cells that infiltrate the corpus callosum (Fig. 1e), normal oligodendrocyte maturation is accompanied by a progressive loss of migratory potential\(^{36,37}\). We therefore sought to understand the impact of differentiation on GBM invasion by imaging whole sections of 4 weeks tumours. Surprisingly, SOX10 OE tumours appeared less diffuse and migrated much shorter distances than controls (Fig. 6e and Supplementary Fig. 6f). In addition, within SOX10 OE tumours the majority of invasive tumour cells had low or undetectable SOX10 levels, whereas SOX10 high cells were found predominantly closest to the tumour bulk (Fig. 6f). These results indicate that differentiation also reduces the migration of GBM cells. To determine whether this phenotype was cell-intrinsic, we measured in vitro motility of SOX10 OE and Control G144 cells by live cell imaging and found that increased SOX10 levels profoundly reduced cell motility (Supplementary Fig. 6g–i). This was not an artefact of SOX10 overexpression, as O4\(^+\) tumour cells acutely FACS-purified from the corpus callosum of wildtype G144 xenografts were also significantly less motile than O4\(^-\) cells from the same region (Fig. 6g).

These results suggest that white matter effects are tumour suppressive and might slow down the progression of the primary disease. To test this hypothesis experimentally, we compared the tumourigenicity of parental and SOX10 knock-out G144 cells, in which differentiation along the oligodendrocyte lineage is abolished (Fig. 5h–j). SOX10 knock-out tumours had a significantly faster growth rate than controls, as measured by longitudinal bioluminescence imaging and analysis of Edu incorporation (Fig. 6h–i). These experiments demonstrate that white matter-driven pre-oligodendrocyte maturation suppresses tumourigenesis by inhibiting proliferation and invasion of GMB cells and that in the absence of this response GBMs are more aggressive.

Finally, we explored the translational potential of these findings by testing whether known myelination-inducing pharmacological agents could differentiate GMB cells by increasing endogenous SOX10 levels. We first treated cultured G144 cells with two compounds: the clinically-approved anti-asthma medication Pranlukast and the cell permeable cAMP analogue dibutylryl cAMP (db-CAMP). In addition to blocking cysteinyl-leukotriene 1 receptor, Pranlukast inhibits GPR17, a negative regulator of oligodendrocyte development\(^{38-40}\). GPR17 is normally expressed in immature oligodendrocytes\(^{38}\) and, importantly, was strongly induced in G144 cells purified from the CC and upon SOX10 overexpression (Figs. 1d and 5b). db-CAMP treatment of normal progenitors results in elevated intracellular cAMP levels, which promote oligodendrocyte differentiation, likely by phenocopying GPCR activity\(^{41,42}\). Treatment of G144 with either compound for 2 weeks was sufficient to increase endogenous SOX10 levels and differentiation to O4\(^+\) pre-oligodendrocyte cells, leading to a reduction in proliferation (Fig. 7a–h). These effects were fully dependent on SOX10, as no differentiation was observed in drug-treated SOX10 knock-out cells (Supplementary Fig. 7a, b). Next, we administered Pranlukast to tumour-bearing mice in vivo. As Pranlukast has poor BBB penetration\(^{43}\), we delivered it intrathecally using osmotic mini-pumps. Strikingly, we found a significant increase in the number of SOX10\(^+\) cells in Pranlukast-treated tumours, which was accompanied by a dramatic reduction in proliferation relative to saline-treated controls (Fig. 7i–l). Pranlukast did not affect endogenous inflammatory glia, indicative of a direct effect on the tumour cells (Supplementary Fig. 7c–e). We conclude that a subset of GBMs can be induced to undergo pre-oligodendrocyte differentiation with small molecules that raise SOX10 levels and propose that such differentiation therapy may be an effective strategy for curtailing tumourigenesis and recurrence.
Discussion

The failure of both conventional and targeted therapies is commonly attributed to the pervasive molecular intra- and inter-tumoural heterogeneity of GBM, which occurs at the genetic, epigenetic, transcriptional and functional levels. Despite this staggering diversity however, recent studies are beginning to reveal that all GBMs converge on a finite number of cellular states, which recapitulate normal developmental programmes. Such convergence offers hope of identifying shared biological vulnerabilities that could be exploited for the treatment of many...
patients, independent of genetic diversity. Here we identified one such vulnerability: the competence of GBM cells to undergo pre-oligodendrocyte differentiation.

Although GSCs retain ability to partially differentiate into mature glial cells in vitro, the extent to which this occurs in tumours has remained unclear.8-10,32. Our results demonstrate the existence of robust differentiation in vivo, including in patient material, which is controlled by the microenvironment. They further reveal that differentiation occurs in specialised niches within the brain and identify the white matter as one such pro-differentiative niche for tumour cells with oligodendrocyte competency.30. Thus, despite the presence of extensive genetic abnormalities, exposure to appropriate environmental cues is sufficient to revert GBM cells to a more normal, differentiated phenotype, underscoring the dominance of the microenvironment in suppressing malignancy33,34.

We found that differentiation is an injury-like response, which results from infiltrating tumour cells disrupting the white matter. Thus, although invasion along white matter is a common mode of infiltration, the ensuing myelin damage creates a tumour-suppressive feedback loop, which paradoxically slows GBM spread. Interestingly, the microenvironment of tumour-infiltrated white matter appeared remarkably similar to that of neuroinflammatory disease states, entailing severe demyelination and axonal pathology.35. Our EM analysis suggests that in the tumour context demyelination may be caused by both oligodendrocyte and neuron death. The underlying mechanisms remain to be determined, but a combination of compression injury to the axons, microglia and astrocytes activation, as well as excitotoxins are all likely to contribute.35,36.

The finding of OPC activation in tumour-infiltrated white matter suggests that the ensuing demyelinating microenvironment initiates a repair programme in normal glia. It is therefore tempting to speculate that GBM cells may recapitulate a similar oligodendrogenic programme, which results in their partial differentiation to pre-oligodendrocyte cells. While it is clear that an injury-like microenvironment drives differentiation, the signals and cell types responsible remain undefined. These are likely to be complex and combinatorial, but our results suggest that microglia, a key regulator of normal re-myelination, may play an important role.37 Indeed, we found that microglia activation was selective to tumour white matter and immediately preceded tumour cell differentiation. It would be of great interest to examine the contribution of microglia to GBM differentiation in future studies.

Our findings are of clinical relevance as we show that exploiting the aberrant GBM injury-like response through niche-independent upregulation of SOX10, locked tumour cells in the differentiated, non-proliferative state and suppressed tumourigenesis in preclinical models of the disease. Furthermore, our work predicts that myelination-promoting compounds, should be particularly effective in tumours with oligodendrocyte lineage competency, providing a potential strategy for patient stratification. Future studies should continue to explore how the
Fig. 7 Myelination-promoting compounds suppress tumour growth. a, representative images of untreated and dibutyryl cAMP-treated (dbcAMP) G144 cultures stained for SOX10 (green), EdU (grey), O4 (red) and DAPI (blue). Scale = 100 µm. b, SOX10 (green) and DAPI (blue) staining of G144 cultures treated with vehicle (Control) or Pranlukast. Scale = 50 µm. c–e, quantification of cultures in a, showing percentages of indicated populations before and after treatment. Fold change relative to control cultures (f.c.) is shown in c and d. ≥1000 cells across duplicate coverslips were counted per biological repeat. Mean ± SEM, n = 3 independent cultures, a p < 0.0001, b p = 0.01, c p = 0.02. Unpaired two-tailed Student’s t test. f–h, quantification of cultures in b, showing percentages of indicated populations before and after treatment. Fold change relative to control cultures (f.c.) is shown in g. ≥800 cells across duplicate coverslips were counted per biological repeat. Mean ± SEM, n = 3 independent cultures. c p = 0.002, d p = 0.05, e, ≥0.006. Unpaired two-tailed Student’s t test. i, j, representative immunofluorescence images of DMSO- (Control) and Pranlukast-treated GFP+ G144 xenografts (PDX144) stained for SOX10 (red) and EdU (red). Scale = 500 µm. k, quantifications of number of SOX10+ and I, EdU+ tumour cells in the xenografts shown in i and j. Scale = 500 µm. Mean ± SEM, n = 3 xenografts. t p = 0.02, u p = 0.002. Unpaired two-tailed Student’s t test.

De novo models. De novo models were generated using a CRISPR/Cas9-based deletion of Nf1, Pten and Trp53 tumour suppressors58, tdTomato encoding piggybac transposons were co-delivered to fluorescently label resulting tumours. Briefly, P2 C57Bl/6 pups underwent intraventricular plasmid administration using an Eppendorf FemtoJet Microinjector. Electroporation was performed using two electrodes positioned on either side of the pup’s head delivering 5 square pulses (100 V, 50 ms pulse ON, 850 msec pulse OFF) delivered by the Gemini BTX electroporator.

Patient-derived xenograft models. Xenografts were performed using CD-1 nude mice for G144 and NOD-SCID-IL2r gamma chain-deficient (NSG) for all other lines. For all tumour studies 8–12 week old female immunocompromised mice underwent stereotactic implantation of GSC lines using 1 × 105 cells with the exception of G144 reinjection experiments for which 5 × 104 were used (anteroposterior 0, mediolateral -2.5, dorsoventral -3.5; outer cortex: anteroposterior 0, mediolateral -1, dorsoventral -1.75; inner cortex: anteroposterior 0, mediolateral -1, dorsoventral -0.5). Tumour growth was monitored using an IVIS Spectrum in vivo imaging system (Perkin Elmer). A total of 10 min following i.p. D-luciferin (120 mg/kg). Intrave neural bioluminescent images were acquired under isoflurane anaesthesia. Tumour size was quantified by calculating total flux (photons/s/cm²) using Living Image software (Xenogen, Caliper Life Sciences). Animals were sacrificed and tumours collected when they showed signs of distress or >10% weight loss. For experiments shown in Fig. 4c–f and r–u tumours were collected at 4 weeks post-implantation. To assess tumour cell proliferation EdU was administered by i.p. injection (50 mg/kg) 4 h prior to collection. Survival was analysed using the Kaplan–Meier method and significance calculated using the log-rank Mantel–Cox test. For all models GBM tumour samples were obtained from CD1 nude mice for G144 and NOD-SCID-IL2r gamma chain-deficient (NSG) for all other lines. Xenografts were performed using CD-1 nude mice for G144 and NOD-SCID-IL2r gamma chain-deficient (NSG) for all other lines.

Methods

Animals. All procedures were performed in compliance with the Animal Scientific Procedures Act, 1986 and approved by the UCL Animal Welfare and Ethical Review Body (AWERB) in accordance with the International guidelines of the Home Office (UK). C57Bl6 and immunocompromised mouse lines were purchased from Charles River.

Patient-derived xenograft models. Xenografts were performed using CD-1 nude mice for G144 and NOD-SCID-IL2r gamma chain-deficient (NSG) for all other lines. For all tumour studies 8–12 week old female immunocompromised mice underwent stereotactic implantation of GSC lines using 1 × 105 cells with the exception of G144 reinjection experiments for which 5 × 104 were used (anteroposterior 0, mediolateral -2.5, dorsoventral -3.5; outer cortex: anteroposterior 0, mediolateral -1, dorsoventral -1.75; inner cortex: anteroposterior 0, mediolateral -1, dorsoventral -0.5). Tumour growth was monitored using an IVIS Spectrum in vivo imaging system (Perkin Elmer). A total of 10 min following i.p. D-luciferin (120 mg/kg). Intrave neural bioluminescent images were acquired under isoflurane anaesthesia. Tumour size was quantified by calculating total flux (photons/s/cm²) using Living Image software (Xenogen, Caliper Life Sciences). Animals were sacrificed and tumours collected when they showed signs of distress or >10% weight loss. For experiments shown in Fig. 4c–f and r–u tumours were collected at 4 weeks post-implantation. To assess tumour cell proliferation EdU was administered by i.p. injection (50 mg/kg) 4 h prior to collection. Survival was analysed using the Kaplan–Meier method and significance calculated using the log-rank Mantel–Cox test. For all models GBM tumour samples were obtained from CD1 nude mice for G144 and NOD-SCID-IL2r gamma chain-deficient (NSG) for all other lines. Xenografts were performed using CD-1 nude mice for G144 and NOD-SCID-IL2r gamma chain-deficient (NSG) for all other lines.
were resuspended in 500 μl GSC media containing 1:500 mouse anti-O4 (Alexa Fluor 594) and incubated for 15 min at 37 °C. Cells were washed once in PBS + 3% BSA and resuspended in 400 μl FACS buffer containing 1×10000 DAPI. O4+ and O4 populations were seeded in serum-free GSC media containing EGF and FGF for live-cell imaging.

**Whole-transcriptome amplification, library construction, sequencing and processing.** For RNA-seq of FACS-sorted tumour cells, ddRNA libraries were prepared according to the Smart-seq2 protocol from FACS-sorted GFP+ cells (REN+). Next-generation sequencing libraries were prepared from 1 ng ddRNA using the Nextera XT DNA library preparation kit (Illumina) and indexed using the Nextera XT index kit (Illumina). For RNA-seq in vitro GSCs, oligo dT-based mRNA isolation was performed using the NEBNext Poly(A) magnetic isolation module. ddRNA libraries were prepared using the NEBNext Ultra II directional RNA library prep kit and indexed using NEBNext Multiplex Oligos. All libraries were diluted to a final concentration of 2.5 nM, pooled and sequenced on an Illumina HiSeq 2500 instrument. Raw data were processed using RTA version 1.18.64, with default filter and quality settings. The reads were demultiplexed with CASAVA 1.8.4 (allowing 0 mismatches). Reads were aligned to the human reference genome (hg38) and assigned to genomic features both using the STAR aligner40. After filtering, only genes with at least 5 counts in at least 3 samples were included in the final dataset (n = 16044 for in vivo RNA-seq, n = 14621 for in vitro RNA-seq).

**Analysis of RNA-seq data.** Differential expression analysis was performed and normalized counts were generated using the DESeq2 Bioconductor package (Supplementary Table 1). For Fig. 1c, genes significantly regulated (P < 0.1) and with an absolute DESeq2 log2 ratio > 0.58 in at least one of the Corpus callosum vs Bulk or Striatum vs Bulk comparisons were selected and clustered using K-means (7 clusters). Overlap of each cluster with gene signatures specific for proliferating cells and a series of brain cell types was assessed using a one-sided Fisher exact test and p-values were corrected for multiple testing using the Benjamini-Hochberg approach. For Supplementary Fig. 1d, genes belonging to each gene signatures and significantly regulated (P < 0.1) in each comparison are shown. Brain signatures were generated using human and mouse transcriptomics datasets26–28. Genes with multiple entries were averaged and low coverage genes filtered out. Condition replicates were averaged, data median centred before the most variable genes between cell types were selected (Coef variation > 0.5). The cut-offs on coefficient variation, CV > 1 for Zhang 2016, CV > 1.5 for Zhang 2014, CV > 0.1 for Cahoy et al. were used to maximise the discrimination between cell types. We defined good cell type discrimination as gene signatures (Supplementary Fig. 1a). The cut-offs on coefficient variation, CV > 0.5 for in vivo RNA-seq, CV > 1 for in vitro RNA-seq.

**Image processing and quantifications.** All image quantifications were carried out using Fiji ImageJ. For analysis of SOX10 expression in infiltrating G144 tumour cells, white matter (corpus callosum) and grey matter regions containing at least 750 HuNu+ cells were defined for quantification across n = 3 mice and SOX10+ and SOX10− human cells counted within these regions. For analysis of EDU incorporation ≥500 HuNu+ cells were quantified for SOX10 expression and EDU positivity across 2 independent ROIs of infiltrated corpus callosum. For analysis of SOX10+/EDU− cells upon implantation into the white matter of the corpus callosum (n = 3) or grey matter of the upper cortex (n = 4), per xenografts were counted. Analysis of SOX10− and SOX10+ tumour cells undergoing proliferation (Ki67+/Ki67−) in patient tissue, ≥300 cells were quantified across 2 independent ROIs for each case n = 4. For quantification of SOX10− tumour cells in contact with white or grey matter, ≥300 cells were quantified across ≥2 ROIs and ≥300 cells were quantified across ≥2 independent ROIs for each case n = 3.

**Quantification of the percentage of differentiated CCI+ tumour cells in white and grey matter.** For analysis of SOX10+/EDU− tumour cells within white or grey matter, ≥300 cells were quantified across ≥2 ROIs and ≥300 cells were quantified across ≥2 independent ROIs for each case n = 3.

**Quantification of the percentage of differentiated SOX10+ tumour cells in areas of high and low myelin disruption.** ≥120 cells were quantified across ≥6 ROIs selected within intact or disrupted white matter for n = 4 xenografts. Myelin disruption was defined as white matter regions containing Neurolamin+ axons and Fluoresmyelin staining intensity ≥25% of contralateral intact regions. For analysis of endogenous oligodenodcytes (SOX10+/HuNu−) within the corpus callosum as a function of endogenous myelinated SOX10−/HuNu− (SOX10+/HuNu−) tumour cells xenograft quantification was carried out across ≥2 ROIs and counting ≥1000 cells per mouse for n = 5 tumours and n = 2 intact control brains. For quantification of the percentage of G144+ tumour cells in contact with axons within these regions a minimum 300 SOX10+ or SOX10− cells were counted across ≥3 independent ROIs for n = 3 tumours. Contact was defined as ≤15 μm from the edge of the nucleus to a Neuro lamin+ axon. For analysis of microglia within the white matter (corpus callosum), grey matter (cortex) or tumour bulk ≥140 cells per region per xenograft were counted. Analysis of endogenous oligodenodcytes was conducted across 5 xenografts and 2 control brains. ≥240 cells were counted across 2 ROIs for each xenograft. Quantiﬁcations of cleaved caspase-3 staining of G144+ tumour cells within the white matter (corpus callosum), grey matter (cortex) or tumour bulk were conducted for ≥200 cells per region per xenograft (n = 3).

**For timecourse analysis of corpus callosum florosmyelin intensity, mean grey values were normalised to the max grey values to account for variation in image intensity.**

**Cell migration assay.** Live cell imaging was performed using either the Zeiss Live Cell Imaging Z.1 of the IncuCyte Zoom. Cells were tracked manually in ImageJ using the ManualTracking plugin, migration distance was calculated and cell trajectories were visualized using the Chemotaxis and Migration Tool provided by Ibidi.

**Quantitative RT-PCR.** RNA was extracted using Trizol Reagent (Sigma). RNA was reverse transcribed using iScript gDNA clear cDNA synthesis kit (Bio-rad) and quantitative PCR was performed using the qPCRBIO SyGreen Mix Lo-Rox (PCR Biosystems). Relative expression values for each gene of interest were obtained by normalizing to GAPDH. Primers used are detailed in Supplementary Table 4.
intensity. For all timepoints a minimum area of 300 µm² was assayed for ≥3 xenografts.

For analysis of SOX10+ EdU+ cells within the invaded region >340 cells were counted per xenograft (n = 3–4). For analysis of microglia within the invaded or injured cortex ≥290 cells were counted.

For quantification of the percentage of GFP+ tumour cells in contact with axons within these regions a minimum 300 SOX10+ or SOX10- cells were quantified across 3 independent cultures per line. For n = 3 tumour lines, contact was defined as ≤1.5 µm from the edge of the nucleus to a Neurofilm+ axon.

For quantifications of the percentage of O4+ pre-oligodendrocyte cells and EdU+ proliferating cells in control and SOX10 transduced cultures of GSC lines ≥200 cells across duplicate cover slips were counted per biological repeat for n = 6 independent cultures per line.

For analysis of differentiation in SOX10 knock-out cells ≥250 cells were counted across duplicate coverslips per repeat. For SOX10 induction in vitro ≥90 cells per group across 2 independent cultures on triplicate coverslips were counted.

For quantifications of the relative proportions of SOX10+, low or high tumour cells at different timepoints (pre-implantation, 4 weeks and survival (11–12 weeks) ≥270 cells were analysed. SOX10 status was assessed using mean grey values of individual nuclei. SOX10- cells were counted as those with a mean grey value less than the background. SOX10 high threshold was defined by measuring the minimum mean grey value of >20 cells showing high SOX10 expression.

Proliferation of control or SOX10 OE tumours was quantified across n = 3 tumours at 4 weeks post-implantation, counting ≥200 cells from 2 ROIs per mouse. Quantification of invasion of n = 3 tumours was performed by measuring the total area occupied by tumour cells and normalising it to the perimeter of the tumour bulk to account for different rates of tumour growth. Invasion was further analysed within SOX10 OE by measuring the distance of SOX10 low, medium and high expressing cells from the tumour bulk. SOX10 low and high threshold intensities were defined as the 25th and 75th percentiles respectively based on analysis of the average intensities across >100 nuclei within the tumour bulk ≥200 cells were quantified per mouse.

For in vitro quantifications of the percentage of O4+ and EdU+ cells following exposure to pranlukast or control, ≥800 cells across duplicate cover slips were counted for n = 3 independent cultures. For dbaMP experiments, ≥900 cells were counted across duplicate coverslips for n = 3 independent cultures.

For in vivo quantifications of the percentage of SOX10+ GFP+ tumour cells in control or pranlukast treated tumours ≥1200 cells were counted. For analysis of proliferation total EdU+ nuclei were normalised to the total GFP + tumour area (n = 3). For analysis of microgla ≥420 cells were counted across n = 3 xenografts.

Targeted electron microscopy. GFP-labelled G144 cells were injected into the striatum of immune-compromised mice and once tumours had developed, brains were perfusion fixed with 4% formaldehyde, and then further immersion fixed for 8 h. Vibrating microtome sections (100 µm) were immunolabelled for Neurofilament and SOX10 and imaged by confocal to map the location of the tumour within the section. For correlative light and electron microscopy (CLEM) of specific SOX10 positive GFP labelled cells, vibratome sections were mapped using the ×20 objective to identify regions of interest. A small asymmetric piece of tissue (<1 mm) were perfusion fixed for CLEM samples, care was taken to ensure that the piece of tissue was fixed for 1 h. Vibrating microtome sections were then processed for electron microscopy essentially as follows. Sections were further treated sequentially with formaldehyde/glutaraldehyde, osmium tetroxide, potassium ferricyanide, osmium tetroxide, thiocarbohydrazide, uranyl acetate and lead acetate prior to dehydration through an ethanol series and embedding in Epoxy resin. For CLEM samples, care was taken to ensure that the piece of tissue was mounted flat, in the correct orientation, to guarantee targeting the cells of interest imaged by confocal microscopy. Serial ultrathin sections (70 nm) were taken using a diamond knife (Diatome) and an ultramicrotome (UC7, Leica) and collected on formvar coated slot grids. Sections were imaged in a transmission electron microscope (T12 Biologic-ThermoFisher) and captured with a CCD camera running TEm software (Morada, Olympus SIS). All EM analysis was conducted on ≥50 axons (n = 3–4). Axons were considered to have decompacted myelin when >15% of the axonal circumference exhibited decompaction of the associated myelin. Degenerating axons were scored as those exhibiting any of the following features: condensed axoplasm, axonal swelling, vacuoles, dark axoplasm. G ratios were calculated by dividing the axonal diameter by the corresponding axonal + myelin sheath diameter. Feret diameters were used to account for the imperfect circularity of axons.

Neuropathological assessment of SOX10 expression in human GBM. SOX10 expression pattern was investigated in brain tissue samples of 26 glioblastoma patients operated on at the National Hospital for Neurology and Neurosurgery, UCHL Hospitals Foundation Trust between 2009 and 2017. None of the patients had radiotherapy or chemotherapy prior to surgery, and they did not have any relevant comorbidities. Patients consent was obtained for the use of all samples. The project received ethical approval from London—Queen Square Research Ethics Committee (08-077). Clinical approval is provided in Supplementary Table 3.

For each case, the resection material was reviewed and the region containing tumour infiltration in the surrounding white matter was selected for the analysis. The resected tissues were immediately fixed in 10% buffered formalin and processed into paraffin blocks using standard methods in the Division of Neuropathology, NHNN.

Statistics. Statistical analysis was performed using GraphPad Prism 7.0. All data are expressed as mean ± SEM. Significance was calculated using 1- or 2-tailed Student’s t test, ANOVA with Bonferroni post-hoc test, Two-way ANOVA with Sidak’s multiple comparisons tests or Pearson’s correlation as indicated in the figure legends. No statistical method was used to predetermine sample size. Sample size was determined based on existing literature and our previous experience. Shapiro–Wilks test was used to confirm the normal distribution of the data.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Unique materials are available to others and can be obtained by contacting the corresponding author. Patient-derived GGCR cell lines are available via the glioma cellular genomics resource: (www.gccg.org.uk). The raw reads sequencing data and unprocessed counts have been deposited in GEO (GSE139261), processed counting data are available in Supplementary data 1–5. Data for all figures can be found in manuscript, in Supplementary Figures and Supplementary Tables, or from the corresponding author upon reasonable request. Source data are provided with this paper.

Received: 12 January 2021; Accepted: 23 February 2021; Published online: 12 April 2021

References
1. Stupp, R. et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N. Engl. J. Med. 352, 987–996 (2005).
2. Weathers, S. P. & Gilbert, M. R. Advances in treating glioblastoma. F1000Prime Rep. 6, 46 (2014).
3. Singh, S. K. et al. Identification of human brain tumour initiating cells. Nature 432, 396–401 (2004).
4. Chen, J. et al. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 488, 522–526 (2012).
5. Lathia, J. D., Mack, S. C., Mulkearns-Hubert, E. E., Valentim, C. L. & Rich, J. N. Cancer stem cells in glioblastoma. Genes Dev. 29, 1203–1217 (2015).
6. Bao, S. et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444, 756–760 (2006).
7. Galli, R. et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res. 64, 7011–7021 (2004).
8. Lan, X. et al. Fate mapping of human glioblastoma reveals an invariant stem cell hierarchy. Nature 549, 227–232 (2017).
9. Nefeli, C. et al. An integrative model of cellular states, plasticity, and genetics for glioblastoma. Cell 178, 835–849 (2019).
10. Park, N. I. et al. ASC1L reorganizes chromatin to direct neuronal fate and suppress tumorigenicity of glioblastoma stem cells. Cell Stem Cell 21, 209–224 (2017).
11. Silva-Vargas, V., Crouch, E. E. & Doetsch, F. Adult neural stem cells and their niche: a dynamic duo during homeostasis, regeneration, and aging. Curr. Opin. Neurobiol. 23, 935–942 (2013).
12. Calabrese, C. et al. A perivascular niche for brain tumor stem cells. Cancer Cell 11, 69–82 (2007).
13. Li, Z. et al. Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. Cell Cycle 15, 501–513 (2009).
14. Hambardzumyan, D. & Bergers, G. Glioblastoma: defining tumor niches. Trends Cancer 1, 252–265 (2015).
15. Piccirillo, S. G. et al. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature 444, 761–765 (2006).
16. Cuddapah, V. A., Robel, S., Watkins, S. & Sontheimer, H. A neurogenic perspective on glioma invasion. Nat. Rev. Neurosci. 15, 455–465 (2014).
17. Vehlow, A. & Cordes, N. Invasion as target for therapy of glioblastoma multiforme. Biochim Biophys. Acta 1836, 236–244 (2013).
18. Molofsky, A. V. et al. Astrocytes and disease: a neurodevelopmental perspective. Genes Dev. 26, 849–891 (2012).
19. Piccirillo, S. G. et al. Distinct pools of cancer stem-like cells coexist within human glioblastomas and display different tumorigenicity and independent genomic evolution. Oncogene 28, 1807–1811 (2009).
20. Molina, J. R., Hayashi, Y., Stephens, C. & Georgescu, M. M. Invasive glioblastoma cells acquire stemness and increased Akt activation. Neoplasia 12, 463–465 (2010).

21. Brooks, J. L. & Parrinello, S. Vascular regulation of glioma stem-like cells: a balancing act. Curr. Opin. Neurol. 47, 8–15 (2017).

22. Hoelzinger, D. B. et al. Gene expression profile of glioblastoma multiforme invasive phenotype points to new therapeutic targets. Neoplasia 7, 7–16 (2005).

23. Blumenfeld, H. Neuroanatomy Through Clinical Cases, Second Edition. Neuroanatomy Through Clinical Cases, Second Edition, 1–1006 (2010).

24. Scherer, H. J. Structural development in gliomas. Am. J. Cancer 34, 333–351 (1938).

25. Pollard, S. M. et al. Glioma stem cell lines expanded in adherent culture have tumorigenic properties and are suitable for chemical and genetic screens. Cell Stem Cell 4, 568–580 (2009).

26. Zhang, Y. et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J. Neurosci. 34, 11929–11947 (2014).

27. Cahoy, J. D. et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J. Neurosci. 28, 264–278 (2008).

28. Zhang, Y. et al. Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. Neuron 89, 37–53 (2016).

29. Wess, M., Reiprich, S. & Wegner, M. Sox8: a cell-intrinsic timer of myelination in glia. Biol. Chem. 394, 1583–1593 (2013).

30. Elbaz, B. & Popko, B. Molecular control of oligodendrocyte development. Trends Neurosci. 42, 263–277 (2019).

31. Azzarelli, R., Simons, B. D. & Philpott, A. The developmental origin of brain tissue stem cell. Trends Pharm. Sci. 27, 987–998 (2016).

32. Turnescu, T. et al. Sox8 and Sox10 jointly maintain myelin gene expression in oligodendrocytes. Glia 66, 279–294 (2018).

33. Horny, J. et al. The transcription factors Sox10 and Myf5 define an essential regulatory network module in differentiating oligodendrocytes. PLoS Genet. 9, e1003907 (2013).

34. Stolt, C. C. et al. Terminal differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10. Genes Dev. 16, 165–170 (2002).

35. Conti, L. et al. Niche-independent symmetrical self-renewal of a mammalian tissue stem cell. PLoS Biol. 3, e283 (2005).

36. Baratte, A. & Fernandes, A. Temporal oligodendrocyte lineage progression: in vitro models of proliferation, differentiation and myelination. Biochim. Biophys. Acta 1843, 1917–1929 (2014).

37. de Castro, F., Bribian, A. & Ortega, M. C. Regulation of oligodendrocyge precursor migration during development, in adulthood and in pathology. Cell Mol. Life Sci. 70, 4355–4368 (2013).

38. Chen, Y. et al. The oligodendrocyte-specific G protein-coupled receptor GPR17 is a cell-intrinsic timer of myelination. Nat. Neurosci. 12, 1398–1406 (2009).

39. Simon, K. et al. The Orphan G protein-coupled receptor GPR17 negatively regulates oligodendrocyte differentiation via Galphai/2 and its downstream effector molecules. J. Biol. Chem. 291, 705–718 (2016).

40. Hennen, S. et al. Decoding signaling and function of the orphan G protein-coupled receptor GPR17 with a small-molecule agonist. Sci. Signal. 6, ra93 (2013).

41. Raible, D. W. & McMorris, F. A. Induction of oligodendrocyte differentiation by activators of adenylate cyclase. J. Neurosci. Res. 27, 43–46 (1990).

42. Mogha, A., D’Rozario, M. & Monk, K. R. G protein-coupled receptors in myelinating glia. Trends Pharmac. Sci. 37, 977–987 (2016).

43. Tang, S. S. et al. Protective effect of pranlukast on Abeta1(1–42)/43-induced cognitive deficits associated with downregulation of cysteinyl leukotriene receptor 1. Int. J. Neuropharmacol. 17, 581–592 (2014).

44. Verhaak, R. G. et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NFI. Cancer Cell 17, 98–110 (2010).

45. Patel, A. P. et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 344, 1396–1401 (2014).

46. Brennan, C. W. et al. The somatic genomic landscape of glioblastoma. Cell 155, 462–477 (2013).

47. Cancer Genome Atlas Research, N. Comprehensive genomic characterization defines human glioma genes and core pathways. Nature 455, 1061–1068 (2008).

48. Sturm, D. et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Cancer Cell 22, 425–437 (2012).

49. Sottoriva, A. et al. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. Proc. Natl. Acad. Sci. USA 110, 4009–4014 (2013).
