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Review

Current Insights into Mesenchymal Signatures in Glioblastoma

Yuji Matsumoto[a,b*§], Tomotsugu Ichikawa[c], Kazuhiko Kurozumi[d], and Isao Date[a]

[a]*Department of Neurological Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama 700-8558, Japan,* [b]*Department of Neurological Surgery, National Hospital Organization Okayama Medical Center, Okayama 701-1192, Japan,*
[c]*Department of Neurological Surgery, Kagawa Prefectural Central Hospital, Takamatsu 760-8557, Japan,*
[d]*Department of Neurosurgery, Hamamatsu University Hospital, Hamamatsu, Shizuoka 431-3192, Japan*

Glioblastoma (GBM) is a fatal primary malignant brain tumor in adults. Despite decades of research, the prognosis for GBM patients is still disappointing. One major reason for the intense therapeutic resistance of GBM is inter- and intra-tumor heterogeneity. GBM-intrinsic transcriptional profiling has suggested the presence of at least three subtypes of GBM: the proneural, classic, and mesenchymal subtypes. The mesenchymal subtype is the most aggressive, and patients with the mesenchymal subtype of primary and recurrent tumors tend to have a worse prognosis compared with patients with the other subtypes. Furthermore, GBM can shift from other subtypes to the mesenchymal subtype over the course of disease progression or recurrence. This phenotypic transition is driven by diverse tumor-intrinsic molecular mechanisms or microenvironmental factors. Thus, better understanding of the plastic nature of mesenchymal transition in GBM is pivotal to developing new therapeutic strategies. In this review, we provide a comprehensive overview of the current understanding of the elements involved in the mesenchymal transition of GBM and discuss future perspectives.

Key words: glioma, glioblastoma, mesenchymal subtype, mesenchymal transition, heterogeneity

Glioblastoma (GBM) is the most lethal primary brain tumor of the central nervous system in adults [1]. Despite the availability of multidisciplinary treatment including surgery, chemotherapy, radiation therapy, and tumor-treating fields therapy, the prognosis of GBM is still dismal, with a median overall survival of less than two years [2-4]. Several clinical trials have been conducted on treatments for GBM, including molecular targeted therapy and immunotherapy, but the antitumor effects observed in these trials have been limited and these treatments have not improved survival [5-9]. GBMs often change their biological characteristics during progression and recurrence, and intra- and inter-tumor heterogeneity is recognized as one of the crucial factors hindering the therapeutic progress of GBM [10, 11].

To better understand the determinants of GBM heterogeneity and treatment resistance, numerous studies have been conducted to classify and characterize the molecular background of GBM on the basis of clinical, genomic, and transcriptomic features. Phillips *et al.* pioneered the definition of three signatures of GBM on the basis of gene expression: the proneural, proliferative, and mesenchymal signatures [12]. The proneural group expresses genes associated with neurogenesis, and younger patients with this subtype show a better prognosis compared with the other subtypes. In contrast, the proliferative and mesenchymal subtypes

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*Corresponding author. Phone: +81-86-235-7336; Fax: +81-86-227-0191
E-mail: y.matsumoto0715@gmail.com (Y. Matsumoto)
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express genes related to cell proliferation and angiogenesis, and elderly patients with these subtypes show a poor prognosis [12]. The Cancer Genome Atlas (TCGA) Research Network identified four transcriptomic subtypes of GBM (proneural, neural, classical, and mesenchymal) on the basis of unsupervised transcriptome analysis of 202 newly diagnosed GBM cases and indicated strong associations with genetic mutations such as *TP53*, *EGFR*, and *NF1* [13]. Gene expression data from three distinct platforms were integrated into one unified data set, and 840 gene signatures were established to classify GBM into the four subtypes. The proneural, neural, classical, and mesenchymal subtypes were characterized by alterations in *PDGFRA* and point mutations in *IDH1*, expression of neuronal marker genes, high levels of *EGFR* amplification, and reduced *NF1* expression caused by a localized hemizygous deletion in the region of 17q11.2 containing the *NF1* gene, respectively. Although the literature indicates that the proneural subtype is associated with better outcomes and the mesenchymal subtype is associated with poor survival [12-16], there was concern that these findings were influenced by the relatively favorable outcomes of *IDH*-mutant GBMs, which are consistently classified as the proneural subtype [17]. TCGA further investigated the correlation between *IDH* wild-type GBM subtypes and the immune microenvironment by unsupervised clustering and defined three subtypes: the proneural, classical, and mesenchymal subtypes [18]. The neural signature was not enriched in any cluster, and the neural subtype was considered to represent normal cell contamination. This result is consistent with other studies in which the neural subtype was not detected [17, 19].

These approaches have been useful for characterizing the molecular diversity of tumor bulk, namely intertumoral heterogeneity, in GBM patients. However, they have a limited view of intratumoral heterogeneity within individual GBM patients. To analyze intratumoral heterogeneity, Patel *et al.* used single-cell RNA sequencing (scRNA-seq) to profile 430 cells from five primary *IDH* wild-type GBMs and found heterogeneous subtype expression in individual cells of single tumors [20]. Darmanis *et al.* performed scRNA-seq on 3589 cells obtained from the tumor core as well as surrounding peripheral tissue in a cohort of four *IDH* wild-type GBM patients and observed heterogeneity in intratumor subtypes [21]. More recently, Neftel *et al.* identified four cell states of *IDH* wild-type GBM on the basis of extensive gene expression analysis using scRNA-seq: the neural-progenitor-like (NPC-like), oligodendrocyte-progenitor-like (OPC-like), astrocyte-like (AC-like), and mesenchymal-like (MES-like) states. The authors found that most tumors contained all four states. The authors further compared the fraction of cells in each of the four cell states in each tumor with the three TCGA subtypes [18] and found that the AC-like and MES-like meta-modules corresponded to the classical and mesenchymal subtypes, respectively, while the OPC-like and NPC-like meta-modules both corresponded to the proneural subtype [22]. These studies suggest that despite the intratumoral subtype heterogeneity within GBMs, the mesenchymal subtype exists at the single-cell transcriptome level in tumor cells.

The existence of tumor-initiating/propagating or cancer stem–like cells within brain tumors has been identified [23-25], and substantial evidence has confirmed that GBM contains stem cell–like tumor-initiating cells called glioblastoma stem cells (GSCs) [26, 27]. GSCs are defined by the cellular capacity to self-renew, initiate tumors upon serial transplantation, and recapitulate tumor cell heterogeneity [26]. The significance of GSCs has been supported by previous studies showing that GSCs promote resistance to conventional therapies, invasion, angiogenesis, and recurrence [28-32]. Recent studies revealed that GSCs can be classified into two mutually distinct subtypes, the proneural or mesenchymal subtype, on the basis of their gene expression profiles and distinct biological characteristics [33-36]. Recently, Richards *et al.* used a combination of scRNA-seq and genome-wide CRISPR screening to characterize the cellular phenotypes of GSCs cultured from GBM patients and found that GSCs mapped along a transcriptional gradient between two cellular states: the Developmental and Injury Response states. Developmental GSCs corresponded to the AC-like, OPC-like, and NPC-like cell states, whereas Injury Response GSCs corresponded to the MES-like state [37].

Although various sets of mesenchymal signature genes have been proposed, multiple studies on the mesenchymal phenotype have indicated that GBM patients with the mesenchymal subtype tend to have shorter survival times than patients with other subtypes when the analysis is restricted to samples with low transcriptional heterogeneity [38-40]. The mesenchymal subtype has been characterized as more aggressive,

invasive, angiogenic, inflammatory, hypoxic, necrotic, and multitherapy resistant compared with the other subtypes of GBMs [13,18,22,41-44]. Importantly, the shift of GBM towards the mesenchymal subtype has been identified to be closely associated with treatment-induced phenotypic changes in recurrence. In addition, the mesenchymal transition has been shown to be driven by genetic abnormalities, the tumor microenvironment and immune cells, and altered energy metabolism. The mesenchymal transition of GBMs is described as analogous to epithelial-mesenchymal transition (EMT), which is a reversible cellular program that plays a crucial role in the malignant progression of many types of cancer [12,45-48]. These findings suggest that understanding the plastic nature of the mesenchymal changes in GBMs is crucial for developing novel therapeutic strategies. Thus, in this review, we will focus on the key factors that affect the course of GBM progression towards the mesenchymal signature.

Transcription Factors and Gene Mutations in Mesenchymal Transition of GBM

In an attempt to identify master transcription factors, Carro *et al.* used reverse-engineering and an unbiased microarray technique to reveal the transcriptional network for the mesenchymal transition of GBMs and found that two transcription factors (C/EBP-β and STAT3) act as the master regulators in the mesenchymal transition [42]. We previously demonstrated that annexin A2 (ANXA2) regulated oncostatin M receptor (OSMR) expression via STAT3 and drove mesenchymal transition in a STAT3-dependent manner in GBMs (the ANXA2–STAT3–OSMR axis) [49] (Fig. 1 and 2). Furthermore, previous studies showed that STAT3 modulated SLUG expression by directly binding to the Slug promotor, inducing the mesenchymal transformation in GSCs [50,51]. In line with these results, STAT3 inhibitors such as AZD1480, ruxolitinib, and HJC0512 were shown to abrogate the mesenchymal transition and exhibited antitumor effects *in vitro* and *in vivo* [52,53].

Another important master regulator of the mesenchymal gene expression signature is TAZ. TAZ and its paralog YAP are transcriptional coactivators, and active YAP/TAZ translocate to the nucleus and interact predominantly with TEAD transcription factors to play diverse roles in cancer-relevant functions via the Hippo pathway [54,55]. Bhat *et al.* revealed that TAZ in a complex with TEAD2 was directly recruited to the gene promoters that encoded proteins that induce the mesenchymal transition and played an essential role in driving the mesenchymal differentiation of malignant

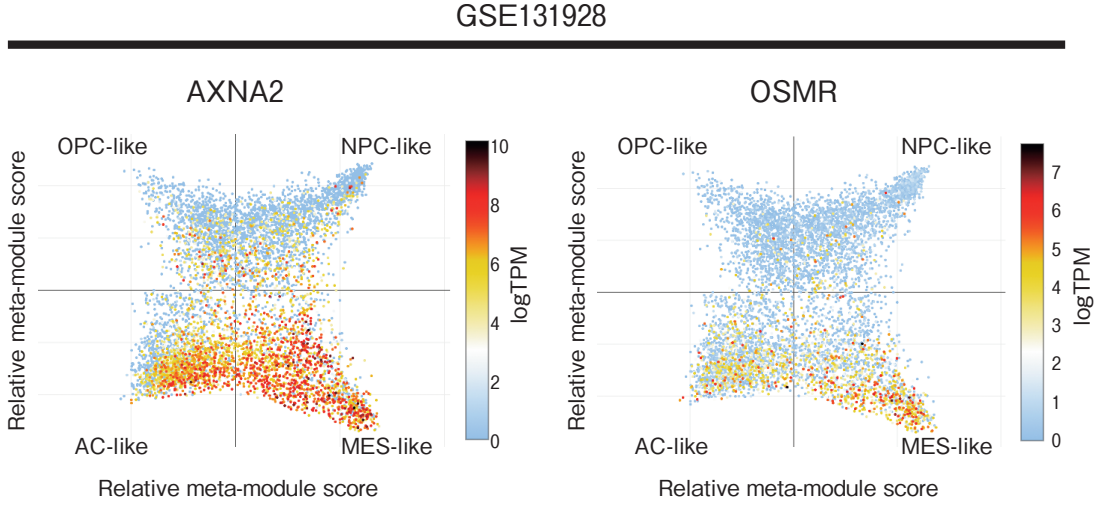

Fig. 1 ANXA2 and OSMR expression in the two-dimensional representation of cellular states. Both ANXA2 and OSMR were enriched in the MES-like state that corresponded to TCGA mesenchymal subtype [22]. Each quadrant represents one cellular state, the exact location of the malignant cells (dots) reflects their relative score of the meta-modules, and the colors reflect the gene expression level. Source data is accessible from the Broad Institute Single-Cell Portal (https://singlecell.broadinstitute.org/single_cell/study/SCP393/single-cell-rna-seq-of-adult-and-pediatric-glioblastoma) and GSE131928 [22] in the NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/). AC, astrocyte; MES, mesenchymal; NPC, neural-progenitor-cell; OPC, oligodendrocyte-progenitor-cell; TPM, transcripts per million.

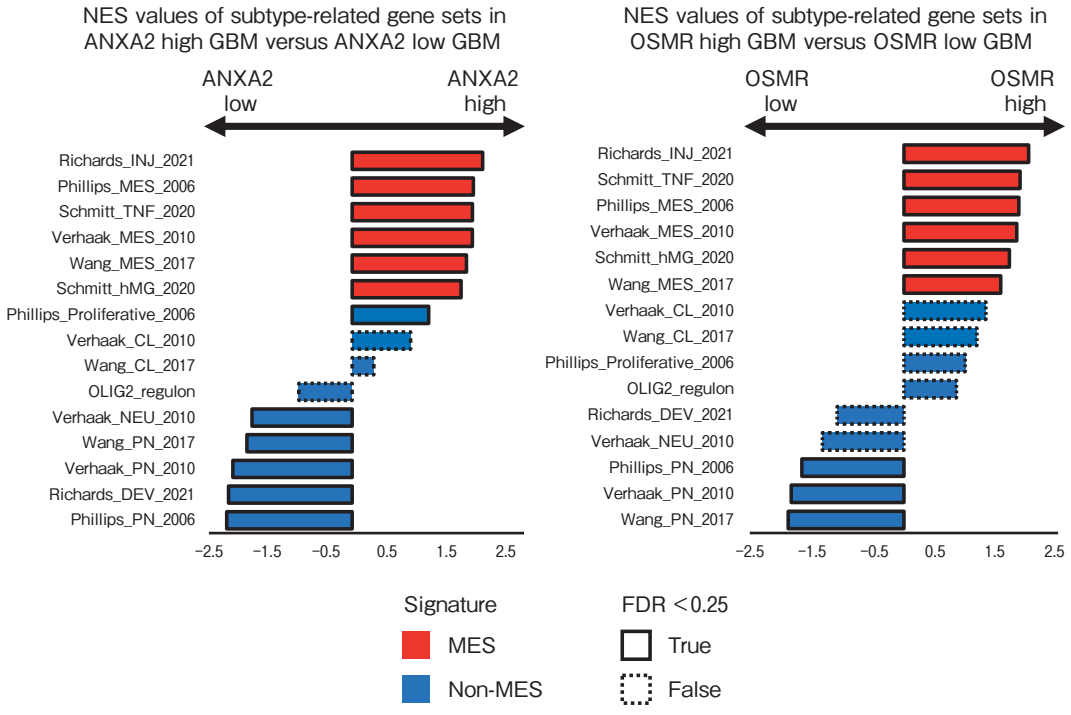

Fig. 2 Gene set enrichment analysis (GSEA) of subtype-related gene sets utilizing the TCGA GBM dataset (n = 538). GSEA of the TCGA GBM dataset revealed significant enrichment of multiple mesenchymal signature gene sets in GBM specimens with high expression of either ANXA2 or OSMR. TCGA GBM dataset was downloaded from GlioVis [158] and analyzed using GSEA [159, 160] (http://www.broadinstitute.org/gsea/index.jsp). FDR was used to determine statistical significance and was considered significant at 0.25 or below, as previously described [160]. The subtype-related gene sets were derived from Marques *et al.* [65]. FDR, false discovery rate; GBM, glioblastoma; MES, mesenchymal; NES, normalized enrichment score; TCGA, The Cancer Genome Atlas.

glioma. Notably, the targets of the mesenchymal network induced by TAZ were relatively nonoverlapping with those driven by STAT3 and C/EBP-β, suggesting that TAZ is an independent modulator of the mesenchymal signatures in GBM [56]. Consistent with this study, Yee *et al.* found that TAZ was significantly and more highly expressed in the mesenchymal subtype than in other subtypes in TCGA GBM dataset and promoted tumor necrosis [57]. More recently, Uneda *et al.* identified that the signature genes of differentiated GBM cells (DGCs) were associated with mesenchymal signature genes in the public GBM datasets, and DGCs showed significantly enriched YAP/TAZ/TEAD expression compared with GSCs [58]. Furthermore, Vigneswaran *et al.* reported that verteporfin, a benzoporphyrin derivative that inhibits YAP/TAZ-TEAD-mediated transcription, induced apoptosis in patient-derived EGFR-amplified/mutant GBM cells and provided significant survival benefit in an orthotopic xenograft GBM model [59].

Intriguingly, NF-κB is closely involved in the complex mesenchymal transcriptional networks. Bhat *et al.* revealed that TNF-α activated NF-κB, and NF-κB regulated three master transcription factors (STAT3, C/EBP-β, and TAZ) to promote mesenchymal differentiation of GSCs and poor radiation response [41]. Yin *et al.* indicated that TNF-α-induced NF-κB activation upregulated TGM2 expression and that TGM2 triggered mesenchymal differentiation of GSCs by regulating these three master transcription factors. The authors found that TGM2 induced the proteasomal degradation of GADD153, a negative regulator of C/EBP-β, and thus upregulated C/EBP-β expression [60]. Furthermore, Iwata *et al.* discovered that TNF-α-induced NF-κB activation increased ICOSLG expression and that ICOSLG expression by mesenchymal GSCs induced IL-10-producing pro-tumorigenic T cells [61]. Regarding the molecular mechanism of NF-kB activation, Kim *et al.* reported that MLK4, a serine-threonine kinase, was an upstream regulator of NF-κB [62].

Genomic abnormalities in the NF1 gene, such as deletions and mutations, have been reported as one of the major hallmarks of mesenchymal GBMs [13,14,18,22,63]. NF1 deficiency has been shown to result in increased recruitment of tumor-associated macrophages to tumor sites [18], and NF1 mutation along with the absence of EGFR amplification and PTEN deletion were strongly correlated with tumor-infiltrating lymphocytes in GBM [64]. Marques *et al.* recently reported that NF1 regulated FOSL1 expression via upregulating RAS/MAPK activity and that FOSL1 played a vital role in the mesenchymal transition, stemness, and tumor growth of GBMs [65].

Effects of Microenvironment Factors on Mesenchymal Signatures of GBM

Sottoriva *et al.* collected four to six tumor fragments from spatially distinct regions within an individual tumor from individual GBM patients to evaluate the regional heterogeneity and identified that each region exhibited the distinct TCGA subtype [66]. Jin *et al.* later identified that tumor cells in the enhancing region highly expressed proneural signature genes, while those in the necrotic region showed high expression of mesenchymal signature genes [44]. Furthermore, Puchalski *et al.* described the anatomically-based comprehensive molecular pathology atlas of glioblastoma that assigned individual histological features to genomic alterations and gene expression patterns. This analysis revealed an apparent correlation between anatomical features and molecular subtypes; the mesenchymal signature predominated in the pseudopalisading cells around necrosis and the microvascular proliferation areas, while the proneural signature predominated in the infiltrating tumor and cellular tumor areas [67]. Minata *et al.* also reported that two distinct types of GSCs exist in the invasive edge and tumor core in GBMs, corresponding to the proneural and mesenchymal subtype, respectively [68]. Indeed, hypoxia is considered a crucial microenvironment factor of the tumor core region of GBMs [11], and GBM cells under the hypoxic and perinecrotic microenvironment showed increased expression of master mesenchymal regulators including C/EBP-β and STAT3 [69]. Furthermore, previous studies demonstrated that the hypoxia-dependent mesenchymal transition was controlled by HIF1α or HIF2α [70-73]. Darmanis *et al.* found that while macrophages were predominant in the tumor core, microglia were the major population at the invasive edge. This distribution contributed to the shaping of a differential microenvironment, in which anti-inflammatory and pro-angiogenic markers were expressed in the tumor core while inflammatory markers were expressed in the tumor periphery [21]. These results demonstrated that the mesenchymal transition of GBM is closely associated with microenvironmental factors.

The majority of the non-neoplastic infiltrates in the GBM microenvironment is macrophages/microglia [74-79]. Previous studies have shown that glioma-associated macrophages/microglia were enriched in mesenchymal GBMs compared with other subtypes and contributed to the mesenchymal transition [18,22,41,77,80-83]. Two recent studies using single-cell analysis demonstrated that MARCO, a scavenger receptor expressed on tumor-associated macrophages, drove a phenotypic transition towards the mesenchymal cellular state of GBMs and correlated with poor clinical outcomes [84,85]. A subpopulation of tumor-associated macrophages characterized by MARCO expression was derived from bone marrow and existed exclusively in *IDH* wild-type GBMs [84]. Gene set enrichment analysis revealed that the expression of pro-inflammatory gene signatures related to IFN-α response, IFN-γ response, allograft rejection, and TNF-α signaling mediated by NF-κB were attenuated in MARCO-expressing macrophages [84]. Hara *et al.* recently reported that macrophages induced the MES-like state in GBM by activating STAT3 through the interaction between macrophage-derived OSM and its receptors OSMR/LIFR in complex with GP130. The authors further found that transition into the MES-like state, in turn, increased expression of a mesenchymal program in macrophages and cytotoxicity of T cells [43]. Gangoso *et al.* revealed that immune attack drove epigenetic changes to reconfigure transcriptional modules in mesenchymal GSCs, conferring an immunosuppressive microenvironment enriched with macrophages and monocytic-myeloid derived suppressor cells in mesenchymal GBMs. The authors proposed a self-reinforcing feedback loop, in which changes in DNA methylation pattern induced by immune attack triggered a myeloid-affiliated transcriptional program in mesenchymal GSCs, resulting in increased recruitment of macrophages, and IFN-γ provided by macrophages in turn promoted epigenetic changes [86]. Dumas *et al.* found that GBM-initiating cells elicited mTOR signaling in the

microglia but not bone marrow–derived macrophages, and mTOR-dependent regulation of STAT3 and NF-κB activity facilitated a pro-inflammatory tumor microenvironment. The mTOR and tumor-associated microglia signatures were most strikingly correlated in the mesenchymal subtype and not in the proneural subgroup of GBMs [87].

A previous study showed that some *IDH* wild-type GBMs harbored a substantial T cell infiltration, although not as high as brain metastases [77]. Rutledge *et al.* demonstrated that tumor-infiltrating lymphocytes were more abundant in the mesenchymal subtype than in other subtypes in GBM and were strongly related to NF1 and RB1 mutations [64]. The differences in the distribution of infiltrating T cell populations (such as cytotoxic T cells, helper T cells, and regulatory T cells) among GBM subtypes have been less conclusive. Kaffes *et al.* reported that the infiltration of T cell populations of helper T cells, cytotoxic T cells, and regulatory T cells is higher in mesenchymal GBM than in other subtypes [82]. Indeed, some studies demonstrated that $CD8^+$ T cells were mainly associated with mesenchymal GBMs [43, 88, 89]. In contrast, Martinez-Lage *et al.* reported that the mesenchymal GBM was enriched in $CD4^+$ T cells compared with other subtypes, with no difference in $CD8^+$ T cells [90]. Thus, further studies are required to elucidate the relationship between the infiltrating T cell population and GBM subtypes.

Effects of Radiotherapy on Mesenchymal Signatures of GBMs

Although current treatment regimens have prolonged the median overall survival of GBM patients, these treatments are still inadequate in terms of efficacy. Radiotherapy has been a key component of GBM treatment for decades and is used in the Stupp regimen [2]. However, the mesenchymal transition has been considered to contribute to radioresistance [18, 41, 68, 91-94]. Bhat *et al.* denoted that a subset of proneural GSCs differentiated into the mesenchymal state in a NF-κB-dependent manner, which in turn elicited an increase in CD44-expressing cells and radioresistance [41]. Moreno *et al.* identified that GPR56, a G-protein-coupled receptor, was enriched in the proneural and classical subtypes of GBMs and lost upon transition to the mesenchymal subtype, and GPR56 inhibited the NF-κB signaling pathway and prevented radioresistance [93]. Furthermore, radiotherapy induced the expression of master mesenchymal regulators such as STAT3 and C/EBP-β and promoted the proneural-to-mesenchymal shift [92]. Goffart *et al.* found that GSCs residing in the subventricular zone (SVZ) exhibit specific resistance to radiation *in vivo*, and these cells have enhanced mesenchymal signatures upregulated by CXCL12 *in vitro* and in the SVZ environment [91]. In addition, Minata *et al.* reported that GSCs enriched for a $CD133^+$ proneural signature at the tumor edge were converted to GSCs enriched for $CD109^+$ mesenchymal signatures, and CD109 activated the YAP/TAZ pathway contributing to radioresistance [68]. Several preclinical studies have been conducted to overcome radiotherapy resistance and radiotherapy-induced mesenchymal transition. The combination of the STAT3 inhibitor such as AZD1480 and ruxolitinib with radiation attenuated the mesenchymal transition and extended survival *in vivo* [52]. YM155, a potent survivin suppressant and radiosensitizer, decreased the mesenchymal signatures of GBM cells, enhanced radiosensitivity, and prevented a radiation-induced invasion by targeting STAT3 [95].

Effects of Chemotherapy on Mesenchymal Signatures of GBM

Temozolomide (TMZ), a DNA alkylating agent, has been widely used as standard chemotherapy for newly diagnosed GBM since the FDA first approved it in 2005, and the Stupp regimen has become widely used [2]. TMZ induces hypermutation or mutagenesis, and these alterations might contribute to the mesenchymal transition [96, 97]. Herting *et al.* established high-grade glioma mouse models corresponding to the mesenchymal subtype by NF1 silencing and the proneural subtype by PDGFB overexpression and found that the NF1-deleted mesenchymal model was less sensitive to both radiation therapy and TMZ than the PDGFB-overexpressing proneural model [98]. Wang *et al.* established TMZ-resistant glioma cells and showed that they exhibited upregulation of mesenchymal signatures mediated by CDC20 [99]. Other studies showed that transcriptional regulators such as FOXO1 or NF1A played a crucial role in TMZ resistance and the mesenchymal transition of GBM cells [100, 101].

Because angiogenesis is one of the hallmarks of GBMs, anti-angiogenic therapies such as bevacizumab, an anti-VEGF monoclonal antibody, were expected to have therapeutic effects. However, phase III studies (AVAglio and RTOG-0825) showed no survival benefit

of bevacizumab for newly diagnosed GBM [5,6]. One possible reason for this insufficient therapeutic effect may be the mesenchymal transition induced by bevacizumab. Piao *et al.* performed gene expression profiling and identified that genes associated with mesenchymal signatures were increased in GBM cells after VEGF treatment compared with untreated controls [102,103]. Other studies revealed that bevacizumab treatment accentuated the mesenchymal transition and conferred resistance to bevacizumab through Wnt/β-catenin signaling, MET activity in a hypoxia-independent manner, ZEB1 expression that altered the metabolic state of bevacizumab-resistant cells via GLUT3 activity, and bevacizumab-induced macroautophagy/autophagy in GSCs [104-108]. Furthermore, a retrospective analysis of the AVAglio trial demonstrated that bevacizumab in combination with standard therapy prolonged PFS over placebo in GBM patients with the mesenchymal and proneural subtype, but prolonged OS was only observed in patients with the proneural subtype [109].

Effects of Recurrence on Mesenchymal Signatures of GBM

Patients with GBM have a poor prognosis as tumor recurrence is inevitable with the current standard of care, and recurrent tumors often have a more aggressive phenotype. [110]. There is currently no standard treatment for patients with recurrent GBM. Numerous regimens, including immunotherapy, antiangiogenic therapy, molecular targeted therapy, reirradiation, stereotactic radiosurgery, and combination therapy, have been investigated in clinical trials [111-113]. However, the prognosis for patients with recurrent GBM remains poor. Thus, elucidating the properties of GBMs at recurrence is crucial for better understanding of the evolution of tumors and improving the treatment of GBMs [114]. Mesenchymal GBMs at tumor recurrence were found to have trends toward a worse overall survival, and reduced expression of mesenchymal signature genes was correlated with more favorable survival and longer time to recurrence in GBM patients [115,116]. Importantly, recurrent GBMs frequently shifted toward the mesenchymal subtype, which was characterized by the loss of OLIG2 expression and the upregulation of YKL40, CD44, STAT3, and VIM expression [12,117]. Furthermore, elevated transcriptional heterogeneity was associated with a higher subtype switching at tumor recurrence, and recurrent GBMs, especially those that underwent the mesenchymal transition, were associated with a higher enrichment of tumor-associated macrophages [18,118]. EGFR amplification or EGFRvIII mutation is a characteristic of the classical subtype of GBM [13,18,116]. Interestingly, van den Bent *et al.* showed that the EGFR amplification status and EGFRvIII expression remained stable in the majority of GBMs evaluated. However, when focusing on EGFRvIII-expressing tumors, approximately half of them lost EGFRvIII expression at recurrence [119]. Cioca *et al.* reported that both primary and recurrent GBMs displayed EGFR expression; approximately 42% of recurrent tumors had reduced EGFR expression compared with primary tumors, and 54% had comparable expression in their cohort [120]. Another study demonstrated that loss of EGFRvIII expression in recurrent tumors was prominently associated with a shift from the classical subtype to other subtypes [116].

Longitudinal transcriptional analysis conducted by TCGA revealed that 45% of *IDH* wild-type GBMs switched subtype upon recurrence [18]. Other studies showed that approximately 30-67% of primary GBMs underwent subtype changes at recurrence [13,116]. The mesenchymal subtype has been considered as the most stable subtype with 45-55% retaining the same subtype at recurrence. However, it is important to note that the frequency of the transition to the mesenchymal subtype was not significant upon tumor recurrence and some tumors shifted to the proneural subtype and others to the classical subtype at recurrence [18,116]. More research is needed to elucidate the molecular biological mechanisms that predispose to the shift toward the mesenchymal subtype upon recurrence.

Effects of the ANXA2–STAT3–OSMR Axis on Mesenchymal Transition of GBM

We previously established two glioma cell lines, J3T-1 and J3T-2, that exhibit distinct invasion patterns and molecular expression and have examined the molecular and pathological phenotypic shifts of GBMs [49,121-124]. We showed that the ANXA2–STAT3–OSMR axis drove the pathological aggressiveness and mesenchymal transition of GBMs [49] (Fig. 3). Consistent with our findings, several studies have reported that ANXA2 promoted tumor aggressiveness and mesenchymal transition in GBMs [125-132]. OSMR and its ligand OSM have been reported to

induce tumorigenesis, tumor aggressiveness, mesenchymal transition, radiotherapy and chemotherapy resistance, shaping an immunosuppressive microenvironment in GBMs [43,133-140]. Notably, OSM is produced by macrophages in the GBM microenvironment and elicited the mesenchymal state in GBM cells via the interaction with its receptors OSMR and LIFR [43,137]. Given that ANXA2 has been reported to exert an effect on macrophage migration to the tumor site and activation [141-145], the ANXA2–STAT3–OSMR axis might drive macrophages to the tumor site and contribute to the development of a tumor ecosystem distinct to GBM. The humanized anti-OSM monoclonal antibody GSK2330811 has recently been validated in a phase II clinical trial to treat diffuse cutaneous systemic sclerosis (NCT03041025), and GSK2330811 exhibited anti-tumor effects in cervical squamous cell carcinoma *in vivo* [146]. Whether GSK2330811 is a new therapeutic approach for GBM by inhibiting the ANXA2–STAT3–OSMR axis should be investigated.

Conclusions and Future Perspectives

In this review, we have discussed the molecular mechanisms related to the mesenchymal transition of GBMs and the clinical significance. There have been significant developments in our understanding of the inter- and intratumoral heterogeneity of GBMs and the interactions with the microenvironment. However, these insights have not yet yielded significant improvements in patient outcomes. One issue is that there is currently no way to know the properties of an individual tumor prior to surgery, making it difficult to promptly tailor treatment strategies on the basis of the characteristics of the tumor. With the development of computational algorithms, artificial intelligence methods are well poised to improve the accuracy of diagnosis and clinical decisions [147]. In particular, machine learning and deep learning methods are being applied to radiomics and radiogenomics. The term radiomics refers to the process of converting images into mineable data and analyzing the data to support decision making, and radiogenomics refers to the process of integrating radiological data with genome-scale data [148,149]. These radiomics and radiogenomics tools have the potential to capture spatial and molecular heterogeneity through non-invasive sampling and to stratify patients into more precise initial diagnostic and therapeutic procedures. In the field of neuro-oncology, radiomics and radiogenomics can predict tumor grade and genetic status including *IDH* mutation and 1p19q-codeletion

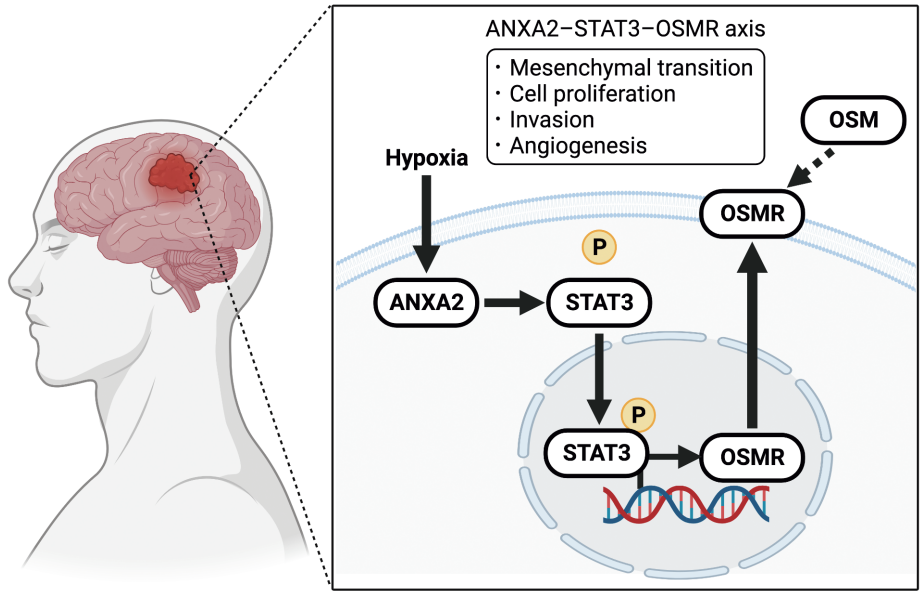

Fig. 3 Schematic diagram of the ANXA2–STAT3–OSMR axis. We previously demonstrated that ANXA2 regulates OSMR expression through STAT3 phosphorylation in GBMs and that this ANXA2–STAT3–OSMR axis drives mesenchymal transition, cell proliferation, invasion, and angiogenesis [49].

status in glioma to clinically useful accuracies [150-157]. Given these advances, the classification of the molecular subtypes of GBM based on imaging alone may soon become a possibility. Comprehensive efforts to further unravel the inter- and intratumoral heterogeneity of GBM and translate these insights into clinical decisions will ultimately contribute to improved patient outcomes.

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References

1. Ostrom QT, Gittleman H, Liao P, Vecchione-Koval T, Wolinsky Y, Kruchko C and Barnholtz-Sloan JS: CBTRUS Statistical Report: Primary brain and other central nervous system tumors diagnosed in the United States in 2010–2014. Neuro Oncol (2017) 19: v1–v88.
2. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E and Mirimanoff RO: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med (2005) 352: 987–996.
3. Stupp R, Taillibert S, Kanner A, Read W, Steinberg D, Lhermitte B, Toms S, Idbaih A, Ahluwalia MS, Fink K, Di Meco F, Lieberman F, Zhu JJ, Stragliotto G, Tran D, Brem S, Hottinger A, Kirson ED, Lavy-Shahaf G, Weinberg U, Kim CY, Paek SH, Nicholas G, Bruna J, Hirte H, Weller M, Palti Y, Hegi ME and Ram Z: Effect of Tumor-Treating Fields Plus Maintenance Temozolomide vs Maintenance Temozolomide Alone on Survival in Patients With Glioblastoma: A Randomized Clinical Trial. JAMA (2017) 318: 2306–2316.
4. Wen PY, Weller M, Lee EQ, Alexander BM, Barnholtz-Sloan JS, Barthel FP, Batchelor TT, Bindra RS, Chang SM, Chiocca EA, Cloughesy TF, DeGroot JF, Galanis E, Gilbert MR, Hegi ME, Horbinski C, Huang RY, Lassman AB, Le Rhun E, Lim M, Mehta MP, Mellinghoff IK, Minniti G, Nathanson D, Platten M, Preusser M, Roth P, Sanson M, Schiff D, Short SC, Taphoorn MJB, Tonn JC, Tsang J, Verhaak RGW, von Deimling A, Wick W, Zadeh G, Reardon DA, Aldape KD and van den Bent MJ: Glioblastoma in adults: a Society for Neuro-Oncology (SNO) and European Society of Neuro-Oncology (EANO) consensus review on current management and future directions. Neuro Oncol (2020) 22: 1073–1113.
5. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, Carpentier AF, Hoang-Xuan K, Kavan P, Cernea D, Brandes AA, Hilton M, Abrey L and Cloughesy T: Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med (2014) 370: 709–722.
6. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, Colman H, Chakravarti A, Pugh S, Won M, Jeraj R, Brown PD, Jaeckle KA, Schiff D, Stieber VW, Brachman DG, Werner-Wasik M, Tremont-Lukats IW, Sulman EP, Aldape KD, Curran WJ, Jr. and Mehta MP: A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med (2014) 370: 699–708.
7. Reardon DA, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, Baehring J, Ahluwalia MS, Roth P, Bähr O, Phuphanich S, Sepulveda JM, De Souza P, Sahebjam S, Carleton M, Tatsuoka K, Taitt C, Zwirtes R, Sampson J and Weller M: Effect of Nivolumab vs Bevacizumab in Patients With Recurrent Glioblastoma: The CheckMate 143 Phase 3 Randomized Clinical Trial. JAMA Oncol (2020) 6: 1003–1010.
8. Stupp R, Hegi ME, Gorlia T, Erridge SC, Perry J, Hong YK, Aldape KD, Lhermitte B, Pietsch T, Grujicic D, Steinbach JP, Wick W, Tarnawski R, Nam DH, Hau P, Weyerbrock A, Taphoorn MJ, Shen CC, Rao N, Thurzo L, Herrlinger U, Gupta T, Kortmann RD, Adamska K, McBain C, Brandes AA, Tonn JC, Schnell O, Wiegel T, Kim CY, Nabors LB, Reardon DA, van den Bent MJ, Hicking C, Markivskyy A, Picard M and Weller M: Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071–22072 study): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol (2014) 15: 1100–1108.
9. Weller M, Butowski N, Tran DD, Recht LD, Lim M, Hirte H, Ashby L, Mechtler L, Goldlust SA, Iwamoto F, Drappatz J, O'Rourke DM, Wong M, Hamilton MG, Finocchiaro G, Perry J, Wick W, Green J, He Y, Turner CD, Yellin MJ, Keler T, Davis TA, Stupp R and Sampson JH: Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. Lancet Oncol (2017) 18: 1373–1385.
10. Aldape K, Brindle KM, Chesler L, Chopra R, Gajjar A, Gilbert MR, Gottardo N, Gutmann DH, Hargrave D, Holland EC, Jones DTW, Joyce JA, Kearns P, Kieran MW, Mellinghoff IK, Merchant M, Pfister SM, Pollard SM, Ramaswamy V, Rich JN, Robinson GW, Rowitch DH, Sampson JH, Taylor MD, Workman P and Gilbertson RJ: Challenges to curing primary brain tumours. Nat Rev Clin Oncol (2019) 16: 509–520.
11. Ichikawa T, Otani Y, Kurozumi K and Date I: Phenotypic Transition as a Survival Strategy of Glioma. Neurol Med Chir (Tokyo) (2016) 56: 387–395.
12. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Soroceanu L, Williams PM, Modrusan Z, Feuerstein BG and Aldape K: Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell (2006) 9: 157–173.
13. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM and Hayes DN: Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell (2010) 17: 98–110.
14. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH, Beroukhim R, Bernard B, Wu CJ, Genovese G, Shmulevich I, Barnholtz-Sloan J, Zou L, Vegesna R, Shukla SA, Ciriello G, Yung WK, Zhang W, Sougnez C, Mikkelsen T, Aldape K, Bigner DD, Van Meir EG, Prados M, Sloan A, Black KL, Eschbacher J, Finocchiaro G, Friedman W, Andrews DW, Guha A, Iacocca M, O'Neill BP, Foltz G, Myers J, Weisenberger DJ, Penny R, Kucherlapati R, Perou CM, Hayes DN, Gibbs R, Marra M, Mills GB, Lander E, Spellman P, Wilson R, Sander C, Weinstein J, Meyerson M, Gabriel S, Laird PW, Haussler D, Getz G and Chin L: The somatic genomic landscape of glioblastoma. Cell (2013) 155: 462–477.
15. Huse JT, Phillips HS and Brennan CW: Molecular subclassification of diffuse gliomas: seeing order in the chaos. Glia (2011) 59: 1190–1199.
16. Zheng S, Chheda MG and Verhaak RG: Studying a complex tumor: potential and pitfalls. Cancer J (2012) 18: 107–114.
17. Sturm D, Witt H, Hovestadt V, Khuong-Quang DA, Jones DT, Konermann C, Pfaff E, Tönjes M, Sill M, Bender S, Kool M, Zapatka M, Becker N, Zucknick M, Hielscher T, Liu XY, Fontebasso AM, Ryzhova M, Albrecht S, Jacob K, Wolter M, Ebinger M, Schuhmann MU, van Meter T, Frühwald MC, Hauch H, Pekrun A, Radlwimmer B, Niehues T, von Komorowski G, Dürken M, Kulozik AE, Madden J, Donson A, Foreman NK, Drissi R, Fouladi M, Scheurlen W, von Deimling A, Monoranu C, Roggendorf W, Herold-Mende C, Unterberg A, Kramm CM, Felsberg J, Hartmann C, Wiestler B, Wick W, Milde T, Witt O, Lindroth AM, Schwartzentruber J, Faury D, Fleming A, Zakrzewska M, Liberski PP, Zakrzewski K, Hauser P, Garami M, Klekner A, Bognar L, Morrissy S, Cavalli F, Taylor MD, van Sluis P, Koster J, Versteeg R, Volckmann R,

Mikkelsen T, Aldape K, Reifenberger G, Collins VP, Majewski J, Korshunov A, Lichter P, Plass C, Jabado N and Pfister SM: Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Cancer Cell (2012) 22: 425–437.
18. Wang Q, Hu B, Hu X, Kim H, Squatrito M, Scarpace L, deCarvalho AC, Lyu S, Li P, Li Y, Barthel F, Cho HJ, Lin YH, Satani N, Martinez-Ledesma E, Zheng S, Chang E, Sauvé CG, Olar A, Lan ZD, Finocchiaro G, Phillips JJ, Berger MS, Gabrusiewicz KR, Wang G, Eskilsson E, Hu J, Mikkelsen T, DePinho RA, Muller F, Heimberger AB, Sulman EP, Nam DH and Verhaak RGW: Tumor Evolution of Glioma-Intrinsic Gene Expression Subtypes Associates with Immunological Changes in the Microenvironment. Cancer Cell (2017) 32: 42–56. e46.
19. Behnan J, Stangeland B, Hosainey SA, Joel M, Olsen TK, Micci F, Glover JC, Isakson P and Brinchmann JE: Differential propagation of stroma and cancer stem cells dictates tumorigenesis and multipotency. Oncogene (2017) 36: 570–584.
20. Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL, Louis DN, Rozenblatt-Rosen O, Suvà ML, Regev A and Bernstein BE: Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science (2014) 344: 1396–1401.
21. Darmanis S, Sloan SA, Croote D, Mignardi M, Chernikova S, Samghababi P, Zhang Y, Neff N, Kowarsky M, Caneda C, Li G, Chang SD, Connolly ID, Li Y, Barres BA, Gephart MH and Quake SR: Single-Cell RNA-Seq Analysis of Infiltrating Neoplastic Cells at the Migrating Front of Human Glioblastoma. Cell Rep (2017) 21: 1399–1410.
22. Neftel C, Laffy J, Filbin MG, Hara T, Shore ME, Rahme GJ, Richman AR, Silverbush D, Shaw ML, Hebert CM, Dewitt J, Gritsch S, Perez EM, Gonzalez Castro LN, Lan X, Druck N, Rodman C, Dionne D, Kaplan A, Bertalan MS, Small J, Pelton K, Becker S, Bonal D, Nguyen QD, Servis RL, Fung JM, Mylvaganam R, Mayr L, Gojo J, Haberler C, Geyeregger R, Czech T, Slavc I, Nahed BV, Curry WT, Carter BS, Wakimoto H, Brastianos PK, Batchelor TT, Stemmer-Rachamimov A, Martinez-Lage M, Frosch MP, Stamenkovic I, Riggi N, Rheinbay E, Monje M, Rozenblatt-Rosen O, Cahill DP, Patel AP, Hunter T, Verma IM, Ligon KL, Louis DN, Regev A, Bernstein BE, Tirosh I and Suvà ML: An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. Cell (2019) 178: 835–849. e821.
23. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, Fiocco R, Foroni C, Dimeco F and Vescovi A: Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res (2004) 64: 7011–7021.
24. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J and Dirks PB: Identification of a cancer stem cell in human brain tumors. Cancer Res (2003) 63: 5821–5828.
25. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD and Dirks PB: Identification of human brain tumour initiating cells. Nature (2004) 432: 396–401.
26. Gimple RC, Bhargava S, Dixit D and Rich JN: Glioblastoma stem cells: lessons from the tumor hierarchy in a lethal cancer. Genes Dev (2019) 33: 591–609.
27. Prager BC, Xie Q, Bao S and Rich JN: Cancer Stem Cells: The Architects of the Tumor Ecosystem. Cell Stem Cell (2019) 24: 41–53.
28. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD and Rich JN: Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature (2006) 444: 756–760.
29. Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, Shi Q, McLendon RE, Bigner DD and Rich JN: Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Res (2006) 66: 7843–7848.
30. Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG and Parada LF: A restricted cell population propagates glioblastoma growth after chemotherapy. Nature (2012) 488: 522–526.
31. Cheng L, Huang Z, Zhou W, Wu Q, Donnola S, Liu JK, Fang X, Sloan AE, Mao Y, Lathia JD, Min W, McLendon RE, Rich JN and Bao S: Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. Cell (2013) 153: 139–152.
32. Wakimoto H, Kesari S, Farrell CJ, Curry WT, Jr., Zaupa C, Aghi M, Kuroda T, Stemmer-Rachamimov A, Shah K, Liu TC, Jeyaretna DS, Debasitis J, Pruszak J, Martuza RL and Rabkin SD: Human glioblastoma-derived cancer stem cells: establishment of invasive glioma models and treatment with oncolytic herpes simplex virus vectors. Cancer Res (2009) 69: 3472–3481.
33. Lottaz C, Beier D, Meyer K, Kumar P, Hermann A, Schwarz J, Junker M, Oefner PJ, Bogdahn U, Wischhusen J, Spang R, Storch A and Beier CP: Transcriptional profiles of CD133+ and CD133− glioblastoma-derived cancer stem cell lines suggest different cells of origin. Cancer Res (2010) 70: 2030–2040.
34. Mao P, Joshi K, Li J, Kim SH, Li P, Santana-Santos L, Luthra S, Chandran UR, Benos PV, Smith L, Wang M, Hu B, Cheng SY, Sobol RW and Nakano I: Mesenchymal glioma stem cells are maintained by activated glycolytic metabolism involving aldehyde dehydrogenase 1A3. Proc Natl Acad Sci U S A (2013) 110: 8644–8649.
35. Spinelli C, Montermini L, Meehan B, Brisson AR, Tan S, Choi D, Nakano I and Rak J: Molecular subtypes and differentiation programmes of glioma stem cells as determinants of extracellular vesicle profiles and endothelial cell-stimulating activities. J Extracell Vesicles (2018) 7: 1490144.
36. Wang L, Babikir H, Müller S, Yagnik G, Shamardani K, Catalan F, Kohanbash G, Alvarado B, Di Lullo E, Kriegstein A, Shah S, Wadhwa H, Chang SM, Phillips JJ, Aghi MK and Diaz AA: The Phenotypes of Proliferating Glioblastoma Cells Reside on a Single Axis of Variation. Cancer Discov (2019) 9: 1708–1719.
37. Richards LM, Whitley OKN, MacLeod G, Cavalli FMG, Coutinho FJ, Jaramillo JE, Svergun N, Riverin M, Croucher DC, Kushida M, Yu K, Guilhamon P, Rastegar N, Ahmadi M, Bhatti JK, Bozek DA, Li N, Lee L, Che C, Luis E, Park NI, Xu Z, Ketela T, Moore RA, Marra MA, Spears J, Cusimano MD, Das S, Bernstein M, Haibe-Kains B, Lupien M, Luchman HA, Weiss S, Angers S, Dirks PB, Bader GD and Pugh TJ: Gradient of Developmental and Injury Response transcriptional states defines functional vulnerabilities underpinning glioblastoma heterogeneity. Nature Cancer (2021) 2: 157–173.
38. Behnan J, Finocchiaro G and Hanna G: The landscape of the mesenchymal signature in brain tumours. Brain (2019) 142: 847–866.
39. Fedele M, Cerchia L, Pegoraro S, Sgarra R and Manfioletti G: Proneural-Mesenchymal Transition: Phenotypic Plasticity to Acquire Multitherapy Resistance in Glioblastoma. Int J Mol Sci (2019) 20: 2746.
40. Kim Y, Varn FS, Park SH, Yoon BW, Park HR, Lee C, Verhaak RGW and Paek SH: Perspective of mesenchymal transformation in glioblastoma. Acta Neuropathol Commun (2021) 9: 50.
41. Bhat KPL, Balasubramaniyan V, Vaillant B, Ezhilarasan R, Hummelink K, Hollingsworth F, Wani K, Heathcock L, James JD, Goodman LD, Conroy S, Long L, Lelic N, Wang S, Gumin J, Raj D, Kodama Y, Raghunathan A, Olar A, Joshi K, Pelloski CE, Heimberger A, Kim SH, Cahill DP, Rao G, Den Dunnen WFA, Boddeke H, Phillips HS, Nakano I, Lang FF, Colman H, Sulman EP and Aldape K: Mesenchymal differentiation mediated by NF-κB promotes radiation resistance in glioblastoma. Cancer Cell (2013) 24: 331–346.
42. Carro MS, Lim WK, Alvarez MJ, Bollo RJ, Zhao X, Snyder EY, Sulman EP, Anne SL, Doetsch F, Colman H, Lasorella A, Aldape K, Califano A and Iavarone A: The transcriptional network for mesenchymal transformation of brain tumours. Nature (2010) 463: 318–325.
43. Hara T, Chanoch-Myers R, Mathewson ND, Myskiw C, Atta L, Bussema L, Eichhorn SW, Greenwald AC, Kinker GS, Rodman C, Gonzalez Castro LN, Wakimoto H, Rozenblatt-Rosen O, Zhuang X, Fan J, Hunter T, Verma IM, Wucherpfennig KW, Regev A, Suvà ML and Tirosh I: Interactions between cancer cells and immune cells drive transitions to mesenchymal-like states in glioblastoma. Cancer Cell (2021) 39: 779–792. e711.
44. Jin X, Kim LJY, Wu Q, Wallace LC, Prager BC, Sanvoranart T, Gimple RC, Wang X, Mack SC, Miller TE, Huang P, Valentim CL, Zhou Q-g,

Barnholtz-Sloan JS, Bao S, Sloan AE and Rich JN: Targeting glioma stem cells through combined BMI1 and EZH2 inhibition. Nature Medicine (2017) 23: 1352–1361.
45. Brabletz T, Kalluri R, Nieto MA and Weinberg RA: EMT in cancer. Nat Rev Cancer (2018) 18: 128–134.
46. Derynck R and Weinberg RA: EMT and Cancer: More Than Meets the Eye. Dev Cell (2019) 49: 313–316.
47. Dongre A and Weinberg RA: New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. Nat Rev Mol Cell Biol (2019) 20: 69–84.
48. Iwadate Y: Epithelial-mesenchymal transition in glioblastoma progression. Oncol Lett (2016) 11: 1615–1620.
49. Matsumoto Y, Ichikawa T, Kurozumi K, Otani Y, Fujimura A, Fujii K, Tomita Y, Hattori Y, Uneda A, Tsuboi N, Kaneda K, Makino K and Date I: Annexin A2-STAT3-Oncostatin M receptor axis drives phenotypic and mesenchymal changes in glioblastoma. Acta Neuropathol Commun (2020) 8: 42.
50. Chesnelong C, Hao X, Cseh O, Wang AY, Luchman HA and Weiss S: SLUG Directs the Precursor State of Human Brain Tumor Stem Cells. Cancers (2019) 11: 1635.
51. Lin J-C, Tsai J-T, Chao T-Y, Ma H-I and Liu W-H: The STAT3/Slug Axis Enhances Radiation-Induced Tumor Invasion and Cancer Stem-like Properties in Radioresistant Glioblastoma. Cancers (2018) 10: 512.
52. Lau J, Ilkhanizadeh S, Wang S, Miroshnikova YA, Salvatierra NA, Wong RA, Schmidt C, Weaver VM, Weiss WA and Persson AI: STAT3 Blockade Inhibits Radiation-Induced Malignant Progression in Glioma. Cancer Res (2015) 75: 4302–4311.
53. Li Z, Zhu T, Xu Y, Wu C, Chen J, Ren Y, Kong L, Sun S, Guo W, Wang Y, Jing C, Dong J, Zhou J, Zhang L, Shen Q and Zhou X: A novel STAT3 inhibitor, HJC0152, exerts potent antitumor activity in glioblastoma. Am J Cancer Res (2019) 9: 699–713.
54. Calses PC, Crawford JJ, Lill JR and Dey A: Hippo Pathway in Cancer: Aberrant Regulation and Therapeutic Opportunities. Trends Cancer (2019) 5: 297–307.
55. Harvey KF, Zhang X and Thomas DM: The Hippo pathway and human cancer. Nature Reviews Cancer (2013) 13: 246–257.
56. Bhat KP, Salazar KL, Balasubramaniyan V, Wani K, Heathcock L, Hollingsworth F, James JD, Gumin J, Diefes KL, Kim SH, Turski A, Azodi Y, Yang Y, Doucette T, Colman H, Sulman EP, Lang FF, Rao G, Copray S, Vaillant BD and Aldape KD: The transcriptional coactivator TAZ regulates mesenchymal differentiation in malignant glioma. Genes Dev (2011) 25: 2594–2609.
57. Yee PP, Wei Y, Kim S-Y, Lu T, Chih SY, Lawson C, Tang M, Liu Z, Anderson B, Thamburaj K, Young MM, Aregawi DG, Glantz MJ, Zacharia BE, Specht CS, Wang H-G and Li W: Neutrophil-induced ferroptosis promotes tumor necrosis in glioblastoma progression. Nature Communications (2020) 11: 5424.
58. Uneda A, Kurozumi K, Fujimura A, Fujii K, Ishida J, Shimazu Y, Otani Y, Tomita Y, Hattori Y, Matsumoto Y, Tsuboi N, Makino K, Hirano S, Kamiya A and Date I: Differentiated glioblastoma cells accelerate tumor progression by shaping the tumor microenvironment via CCN1-mediated macrophage infiltration. Acta Neuropathol Commun (2021) 9: 29.
59. Vigneswaran K, Boyd NH, Oh SY, Lallani S, Boucher A, Neill SG, Olson JJ and Read RD: YAP/TAZ Transcriptional Coactivators Create Therapeutic Vulnerability to Verteporfin in EGFR-mutant Glioblastoma. Clin Cancer Res (2021) 27: 1553–1569.
60. Yin J, Oh YT, Kim JY, Kim SS, Choi E, Kim TH, Hong JH, Chang N, Cho HJ, Sa JK, Kim JC, Kwon HJ, Park S, Lin W, Nakano I, Gwak HS, Yoo H, Lee SH, Lee J, Kim JH, Kim SY, Nam DH, Park MJ and Park JB: Transglutaminase 2 Inhibition Reverses Mesenchymal Transdifferentiation of Glioma Stem Cells by Regulating C/EBPβ Signaling. Cancer Res (2017) 77: 4973–4984.
61. Iwata R, Hyoung Lee J, Hayashi M, Dianzani U, Ofune K, Maruyama M, Oe S, Ito T, Hashiba T, Yoshimura K, Nonaka M, Nakano Y, Norian L, Nakano I and Asai A: ICOSLG-mediated regulatory T-cell expansion and IL-10 production promote progression of glioblastoma. Neuro Oncol (2020) 22: 333–344.
62. Kim SH, Ezhilarasan R, Phillips E, Gallego-Perez D, Sparks A, Taylor D, Ladner K, Furuta T, Sabit H, Chhipa R, Cho JH, Mohyeldin A, Beck S, Kurozumi K, Kuroiwa T, Iwata R, Asai A, Kim J, Sulman EP, Cheng SY, Lee LJ, Nakada M, Guttridge D, DasGupta B, Goidts V, Bhat KP and Nakano I: Serine/Threonine Kinase MLK4 Determines Mesenchymal Identity in Glioma Stem Cells in an NF-κB-dependent Manner. Cancer Cell (2016) 29: 201–213.
63. Ozawa T, Riester M, Cheng YK, Huse JT, Squatrito M, Helmy K, Charles N, Michor F and Holland EC: Most human non-GCIMP glioblastoma subtypes evolve from a common proneural-like precursor glioma. Cancer Cell (2014) 26: 288–300.
64. Rutledge WC, Kong J, Gao J, Gutman DA, Cooper LA, Appin C, Park Y, Scarpace L, Mikkelsen T, Cohen ML, Aldape KD, McLendon RE, Lehman NL, Miller CR, Schniederjan MJ, Brennan CW, Saltz JH, Moreno CS and Brat DJ: Tumor-infiltrating lymphocytes in glioblastoma are associated with specific genomic alterations and related to transcriptional class. Clin Cancer Res (2013) 19: 4951–4960.
65. Marques C, Unterkircher T, Kroon P, Oldrini B, Izzo A, Dramaretska Y, Ferrarese R, Kling E, Schnell O, Nelander S, Wagner EF, Bakiri L, Gargiulo G, Carro MS and Squatrito M: NF1 regulates mesenchymal glioblastoma plasticity and aggressiveness through the AP-1 transcription factor FOSL1. Elife (2021) 10.
66. Sottoriva A, Spiteri I, Piccirillo SG, Touloumis A, Collins VP, Marioni JC, Curtis C, Watts C and Tavaré S: Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. Proc Natl Acad Sci U S A (2013) 110: 4009–4014.
67. Puchalski RB, Shah N, Miller J, Dalley R, Nomura SR, Yoon JG, Smith KA, Lankerovich M, Bertagnolli D, Bickley K, Boe AF, Brouner K, Butler S, Caldejon S, Chapin M, Datta S, Dee N, Desta T, Dolbeare T, Dotson N, Ebbert A, Feng D, Feng X, Fisher M, Gee G, Goldy J, Gourley L, Gregor BW, Gu G, Hejazinia N, Hohmann J, Hothi P, Howard R, Joines K, Kriedberg A, Kuan L, Lau C, Lee F, Lee H, Lemon T, Long F, Mastan N, Mott E, Murthy C, Ngo K, Olson E, Reding M, Riley Z, Rosen D, Sandman D, Shapovalova N, Slaughterbeck CR, Sodt A, Stockdale G, Szafer A, Wakeman W, Wohnoutka PE, White SJ, Marsh D, Rostomily RC, Ng L, Dang C, Jones A, Keogh B, Gittleman HR, Barnholtz-Sloan JS, Cimino PJ, Uppin MS, Keene CD, Farrokhi FR, Lathia JD, Berens ME, Iavarone A, Bernard A, Lein E, Phillips JW, Rostad SW, Cobbs C, Hawrylycz MJ and Foltz GD: An anatomic transcriptional atlas of human glioblastoma. Science (2018) 360: 660–663.
68. Minata M, Audia A, Shi J, Lu S, Bernstock J, Pavlyukov MS, Das A, Kim SH, Shin YJ, Lee Y, Koo H, Snigdha K, Waghmare I, Guo X, Mohyeldin A, Gallego-Perez D, Wang J, Chen D, Cheng P, Mukheef F, Contreras M, Reyes JF, Vaillant B, Sulman EP, Cheng SY, Markert JM, Tannous BA, Lu X, Kango-Singh M, Lee LJ, Nam DH, Nakano I and Bhat KP: Phenotypic Plasticity of Invasive Edge Glioma Stem-like Cells in Response to Ionizing Radiation. Cell Rep (2019) 26: 1893–1905. e1897.
69. Cooper LA, Gutman DA, Chisolm C, Appin C, Kong J, Rong Y, Kurc T, Van Meir EG, Saltz JH, Moreno CS and Brat DJ: The tumor microenvironment strongly impacts master transcriptional regulators and gene expression class of glioblastoma. Am J Pathol (2012) 180: 2108–2119.
70. Heiland DH, Gaebelein A, Börries M, Wörner J, Pompe N, Franco P, Heynckes S, Bartholomae M, hAilín D, Carro MS, Prinz M, Weber S, Mader I, Delev D and Schnell O: Microenvironment-Derived Regulation of HIF Signaling Drives Transcriptional Heterogeneity in Glioblastoma Multiforme. Mol Cancer Res (2018) 16: 655–668.
71. Joseph JV, Conroy S, Pavlov K, Sontakke P, Tomar T, Eggens-Meijer E, Balasubramaniyan V, Wagemakers M, den Dunnen WF and Kruyt FA: Hypoxia enhances migration and invasion in glioblastoma by promoting a mesenchymal shift mediated by the HIF1α-ZEB1 axis. Cancer Lett (2015) 359: 107–116.
72. Qiu W, Song S, Chen W, Zhang J, Yang H and Chen Y: Hypoxia-induced EPHB2 promotes invasive potential of glioblastoma. Int J Clin Exp Pathol (2019) 12: 539–548.

73. Torres Á, Erices JI, Sanchez F, Ehrenfeld P, Turchi L, Virolle T, Uribe D, Niechi I, Spichiger C, Rocha JD, Ramirez M, Salazar-Onfray F, San Martín R and Quezada C: Extracellular adenosine promotes cell migration/invasion of Glioblastoma Stem-like Cells through A(3) Adenosine Receptor activation under hypoxia. Cancer Lett (2019) 446: 112–122.
74. Arrieta VA, Najem H, Petrosyan E, Lee-Chang C, Chen P, Sonabend AM and Heimberger AB: The Eclectic Nature of Glioma-Infiltrating Macrophages and Microglia. Int J Mol Sci (2021) 22.
75. Broekman ML, Maas SLN, Abels ER, Mempel TR, Krichevsky AM and Breakefield XO: Multidimensional communication in the microenvirons of glioblastoma. Nat Rev Neurol (2018) 14: 482–495.
76. Hambardzumyan D, Gutmann DH and Kettenmann H: The role of microglia and macrophages in glioma maintenance and progression. Nat Neurosci (2016) 19: 20–27.
77. Klemm F, Maas RR, Bowman RL, Kornete M, Soukup K, Nassiri S, Brouland JP, Iacobuzio-Donahue CA, Brennan C, Tabar V, Gutin PH, Daniel RT, Hegi ME and Joyce JA: Interrogation of the Microenvironmental Landscape in Brain Tumors Reveals Disease-Specific Alterations of Immune Cells. Cell (2020) 181: 1643–1660. e1617.
78. Li W and Graeber MB: The molecular profile of microglia under the influence of glioma. Neuro Oncol (2012) 14: 958–978.
79. Quail DF and Joyce JA: The Microenvironmental Landscape of Brain Tumors. Cancer Cell (2017) 31: 326–341.
80. Engler JR, Robinson AE, Smirnov I, Hodgson JG, Berger MS, Gupta N, James CD, Molinaro A and Phillips JJ: Increased microglia/macrophage gene expression in a subset of adult and pediatric astrocytomas. PLoS One (2012) 7: e43339.
81. Gabrusiewicz K, Rodriguez B, Wei J, Hashimoto Y, Healy LM, Maiti SN, Thomas G, Zhou S, Wang Q, Elakkad A, Liebelt BD, Yaghi NK, Ezhilarasan R, Huang N, Weinberg JS, Prabhu SS, Rao G, Sawaya R, Langford LA, Bruner JM, Fuller GN, Bar-Or A, Li W, Colen RR, Curran MA, Bhat KP, Antel JP, Cooper LJ, Sulman EP and Heimberger AB: Glioblastoma-infiltrated innate immune cells resemble M0 macrophage phenotype. JCI Insight (2016) 1.
82. Kaffes I, Szulzewsky F, Chen Z, Herting CJ, Gabanic B, Velázquez Vega JE, Shelton J, Switchenko JM, Ross JL, McSwain LF, Huse JT, Westermark B, Nelander S, Forsberg-Nilsson K, Uhrbom L, Maturi NP, Cimino PJ, Holland EC, Kettenmann H, Brennan CW, Brat DJ and Hambardzumyan D: Human Mesenchymal glioblastomas are characterized by an increased immune cell presence compared to Proneural and Classical tumors. Oncoimmunology (2019) 8: e1655360.
83. Schmitt MJ, Company C, Dramaretska Y, Barozzi I, Göhrig A, Kertalli S, Großmann M, Naumann H, Sanchez-Bailon MP, Hulsman D, Glass R, Squatrito M, Serresi M and Gargiulo G: Phenotypic Mapping of Pathologic Cross-Talk between Glioblastoma and Innate Immune Cells by Synthetic Genetic Tracing. Cancer Discov (2021) 11: 754–777.
84. Chen AX, Gartrell RD, Zhao J, Upadhyayula PS, Zhao W, Yuan J, Minns HE, Dovas A, Bruce JN, Lasorella A, Iavarone A, Canoll P, Sims PA and Rabadan R: Single-cell characterization of macrophages in glioblastoma reveals MARCO as a mesenchymal pro-tumor marker. Genome Med (2021) 13: 88.
85. Sa JK, Chang N, Lee HW, Cho HJ, Ceccarelli M, Cerulo L, Yin J, Kim SS, Caruso FP, Lee M, Kim D, Oh YT, Lee Y, Her NG, Min B, Kim HJ, Jeong DE, Kim HM, Kim H, Chung S, Woo HG, Lee J, Kong DS, Seol HJ, Lee JI, Kim J, Park WY, Wang Q, Sulman EP, Heimberger AB, Lim M, Park JB, Iavarone A, Verhaak RGW and Nam DH: Transcriptional regulatory networks of tumor-associated macrophages that drive malignancy in mesenchymal glioblastoma. Genome Biol (2020) 21: 216.
86. Gangoso E, Southgate B, Bradley L, Rus S, Galvez-Cancino F, McGivern N, Güç E, Kapourani CA, Byron A, Ferguson KM, Alfazema N, Morrison G, Grant V, Blin C, Sou I, Marques-Torrejon MA, Conde L, Parrinello S, Herrero J, Beck S, Brandner S, Brennan PM, Bertone P, Pollard JW, Quezada SA, Sproul D, Frame MC, Serrels A and Pollard SM: Glioblastomas acquire myeloid-affiliated transcriptional programs via epigenetic immunoediting to elicit immune evasion. Cell (2021) 184: 2454–2470. e2426.
87. Dumas AA, Pomella N, Rosser G, Guglielmi L, Vinel C, Millner TO, Rees J, Aley N, Sheer D, Wei J, Marisetty A, Heimberger AB, Bowman RL, Brandner S, Joyce JA and Marino S: Microglia promote glioblastoma via mTOR-mediated immunosuppression of the tumour microenvironment. Embo J (2020) 39: e103790.
88. Beier CP, Kumar P, Meyer K, Leukel P, Bruttel V, Aschenbrenner I, Riemenschneider MJ, Fragoulis A, Rümmele P, Lamszus K, Schulz JB, Weis J, Bogdahn U, Wischhusen J, Hau P, Spang R and Beier D: The cancer stem cell subtype determines immune infiltration of glioblastoma. Stem Cells Dev (2012) 21: 2753–2761.
89. Prins RM, Soto H, Konkankit V, Odesa SK, Eskin A, Yong WH, Nelson SF and Liau LM: Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. Clin Cancer Res (2011) 17: 1603–1615.
90. Martinez-Lage M, Lynch TM, Bi Y, Cocito C, Way GP, Pal S, Haller J, Yan RE, Ziober A, Nguyen A, Kandpal M, O'Rourke DM, Greenfield JP, Greene CS, Davuluri RV and Dahmane N: Immune landscapes associated with different glioblastoma molecular subtypes. Acta Neuropathol Commun (2019) 7: 203.
91. Goffart N, Lombard A, Lallemand F, Kroonen J, Nassen J, Di Valentin E, Berendsen S, Dedobbeleer M, Willems E, Robe P, Bours V, Martin D, Martinive P, Maquet P and Rogister B: CXCL12 mediates glioblastoma resistance to radiotherapy in the subventricular zone. Neuro Oncol (2017) 19: 66–77.
92. Halliday J, Helmy K, Pattwell SS, Pitter KL, LaPlant Q, Ozawa T and Holland EC: In vivo radiation response of proneural glioma characterized by protective p53 transcriptional program and proneural-mesenchymal shift. Proc Natl Acad Sci U S A (2014) 111: 5248–5253.
93. Moreno M, Pedrosa L, Paré L, Pineda E, Bejarano L, Martínez J, Balasubramaniyan V, Ezhilarasan R, Kallarackal N, Kim SH, Wang J, Audia A, Conroy S, Marin M, Ribalta T, Pujol T, Herreros A, Tortosa A, Mira H, Alonso MM, Gómez-Manzano C, Graus F, Sulman EP, Piao X, Nakano I, Prat A, Bhat KP and de la Iglesia N: GPR56/ADGRG1 Inhibits Mesenchymal Differentiation and Radioresistance in Glioblastoma. Cell Rep (2017) 21: 2183–2197.
94. Segerman A, Niklasson M, Haglund C, Bergström T, Jarvius M, Xie Y, Westermark A, Sönmez D, Hermansson A, Kastemar M, Naimaie-Ali Z, Nyberg F, Berglund M, Sundström M, Hesselager G, Uhrbom L, Gustafsson M, Larsson R, Fryknäs M, Segerman B and Westermark B: Clonal Variation in Drug and Radiation Response among Glioma-Initiating Cells Is Linked to Proneural-Mesenchymal Transition. Cell Rep (2016) 17: 2994–3009.
95. Zhang X, Wang X, Xu R, Ji J, Xu Y, Han M, Wei Y, Huang B, Chen A, Zhang Q, Li W, Wang J, Li X and Qiu C: YM155 decreases radiation-induced invasion and reverses epithelial-mesenchymal transition by targeting STAT3 in glioblastoma. J Transl Med (2018) 16: 79.
96. Barthel FP, Johnson KC, Varn FS, Moskalik AD, Tanner G, Kocakavuk E, Anderson KJ, Abiola O, Aldape K, Alfaro KD, Alpar D, Amin SB, Ashley DM, Bandopadhayay P, Barnholtz-Sloan JS, Beroukhim R, Bock C, Brastianos PK, Brat DJ, Brodbelt AR, Bruns AF, Bulsara KR, Chakrabarty A, Chakravarti A, Chuang JH, Claus EB, Cochran EJ, Connelly J, Costello JF, Finocchiaro G, Fletcher MN, French PJ, Gan HK, Gilbert MR, Gould PV, Grimmer MR, Iavarone A, Ismail A, Jenkinson MD, Khasraw M, Kim H, Kouwenhoven MCM, LaViolette PS, Li M, Lichter P, Ligon KL, Lowman AK, Malta TM, Mazor T, McDonald KL, Molinaro AM, Nam DH, Nayyar N, Ng HK, Ngan CY, Niclou SP, Niers JM, Noushmehr H, Noorbakhsh J, Ormond DR, Park CK, Poisson LM, Rabadan R, Radlwimmer B, Rao G, Reifenberger G, Sa JK, Schuster M, Shaw BL, Short SC, Smitt PAS, Sloan AE, Smits M, Suzuki H, Tabatabai G, Van Meir EG, Watts C, Weller M, Wesseling P, Westerman BA, Widhalm G, Woehrer A, Yung WKA, Zadeh G, Huse JT, De Groot JF, Stead LF and Verhaak RGW: Longitudinal molecular trajectories of diffuse glioma in adults. Nature (2019) 576: 112–120.
97. Johnson BE, Mazor T, Hong C, Barnes M, Aihara K, McLean CY, Fouse SD, Yamamoto S, Ueda H, Tatsuno K, Asthana S, Jalbert LE,

Nelson SJ, Bollen AW, Gustafson WC, Charron E, Weiss WA, Smirnov IV, Song JS, Olshen AB, Cha S, Zhao Y, Moore RA, Mungall AJ, Jones SJM, Hirst M, Marra MA, Saito N, Aburatani H, Mukasa A, Berger MS, Chang SM, Taylor BS and Costello JF: Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science (2014) 343: 189–193.
98. Herting CJ, Chen Z, Pitter KL, Szulzewsky F, Kaffes I, Kaluzova M, Park JC, Cimino PJ, Brennan C, Wang B and Hambardzumyan D: Genetic driver mutations define the expression signature and microenvironmental composition of high-grade gliomas. Glia (2017) 65: 1914–1926.
99. Wang J, Zhou F, Li Y, Li Q, Wu Z, Yu L, Yuan F, Liu J, Tian Y, Cao Y, Zhao Y and Zheng Y: Cdc20 overexpression is involved in temozolomide-resistant glioma cells with epithelial-mesenchymal transition. Cell Cycle (2017) 16: 2355–2365.
100. Chen C, Han G, Li Y, Yue Z, Wang L and Liu J: FOXO1 associated with sensitivity to chemotherapy drugs and glial-mesenchymal transition in glioma. J Cell Biochem (2019) 120: 882–893.
101. Yu X, Wang M, Zuo J, Wahafu A, Mao P, Li R, Wu W, Xie W and Wang J: Nuclear factor I A promotes temozolomide resistance in glioblastoma via activation of nuclear factor κB pathway. Life Sci (2019) 236: 116917.
102. Piao Y, Liang J, Holmes L, Henry V, Sulman E and de Groot JF: Acquired resistance to anti-VEGF therapy in glioblastoma is associated with a mesenchymal transition. Clin Cancer Res (2013) 19: 4392–4403.
103. Piao Y, Liang J, Holmes L, Zurita AJ, Henry V, Heymach JV and de Groot JF: Glioblastoma resistance to anti-VEGF therapy is associated with myeloid cell infiltration, stem cell accumulation, and a mesenchymal phenotype. Neuro Oncol (2012) 14: 1379–1392.
104. Chandra A, Jahangiri A, Chen W, Nguyen AT, Yagnik G, Pereira MP, Jain S, Garcia JH, Shah SS, Wadhwa H, Joshi RS, Weiss J, Wolf KJ, Lin JG, Müller S, Rick JW, Diaz AA, Gilbert LA, Kumar S and Aghi MK: Clonal ZEB1-Driven Mesenchymal Transition Promotes Targetable Oncologic Antiangiogenic Therapy Resistance. Cancer Res (2020) 80: 1498–1511.
105. Huang W, Zhang C, Cui M, Niu J and Ding W: Inhibition of Bevacizumab-induced Epithelial-Mesenchymal Transition by BATF2 Overexpression Involves the Suppression of Wnt/β-Catenin Signaling in Glioblastoma Cells. Anticancer Res (2017) 37: 4285–4294.
106. Lu KV, Chang JP, Parachoniak CA, Pandika MM, Aghi MK, Meyronet D, Isachenko N, Fouse SD, Phillips JJ, Cheresh DA, Park M and Bergers G: VEGF inhibits tumor cell invasion and mesenchymal transition through a MET/VEGFR2 complex. Cancer Cell (2012) 22: 21–35.
107. Okuda T, Tasaki T, Nakata S, Yamashita K, Yoshioka H, Izumoto S, Kato A and Fujita M: Efficacy of Combination Therapy with MET and VEGF Inhibitors for MET-overexpressing Glioblastoma. Anticancer Res (2017) 37: 3871–3876.
108. Wu HB, Yang S, Weng HY, Chen Q, Zhao XL, Fu WJ, Niu Q, Ping YF, Wang JM, Zhang X, Yao XH and Bian XW: Autophagy-induced KDR/VEGFR-2 activation promotes the formation of vasculogenic mimicry by glioma stem cells. Autophagy (2017) 13: 1528–1542.
109. Sandmann T, Bourgon R, Garcia J, Li C, Cloughesy T, Chinot OL, Wick W, Nishikawa R, Mason W, Henriksson R, Saran F, Lai A, Moore N, Kharbanda S, Peale F, Hegde P, Abrey LE, Phillips HS and Bais C: Patients With Proneural Glioblastoma May Derive Overall Survival Benefit From the Addition of Bevacizumab to First-Line Radiotherapy and Temozolomide: Retrospective Analysis of the AVAglio Trial. J Clin Oncol (2015) 33: 2735–2744.
110. Osuka S and Van Meir EG: Overcoming therapeutic resistance in glioblastoma: the way forward. J Clin Invest (2017) 127: 415–426.
111. Birzu C, French P, Caccese M, Cerretti G, Idbaih A, Zagonel V and Lombardi G: Recurrent Glioblastoma: From Molecular Landscape to New Treatment Perspectives. Cancers (Basel) (2020) 13.
112. Minniti G, Niyazi M, Alongi F, Navarria P and Belka C: Current status and recent advances in reirradiation of glioblastoma. Radiation Oncology (2021) 16: 36.
113. Schritz A, Aouali N, Fischer A, Dessenne C, Adams R, Berchem G, Huiart L and Schmitz S: Systematic review and network meta-analysis of the efficacy of existing treatments for patients with recurrent glioblastoma. Neuro-Oncology Advances (2021) 3.
114. Campos B, Olsen LR, Urup T and Poulsen HS: A comprehensive profile of recurrent glioblastoma. Oncogene (2016) 35: 5819–5825.
115. Cheng WY, Kandel JJ, Yamashiro DJ, Canoll P and Anastassiou D: A multi-cancer mesenchymal transition gene expression signature is associated with prolonged time to recurrence in glioblastoma. PLoS One (2012) 7: e34705.
116. Wang J, Cazzato E, Ladewig E, Frattini V, Rosenbloom DI, Zairis S, Abate F, Liu Z, Elliott O, Shin YJ, Lee JK, Lee IH, Park WY, Eoli M, Blumberg AJ, Lasorella A, Nam DH, Finocchiaro G, Iavarone A and Rabadan R: Clonal evolution of glioblastoma under therapy. Nat Genet (2016) 48: 768–776.
117. Wood MD, Reis GF, Reuss DE and Phillips JJ: Protein Analysis of Glioblastoma Primary and Posttreatment Pairs Suggests a Mesenchymal Shift at Recurrence. J Neuropathol Exp Neurol (2016) 75: 925–935.
118. Lu-Emerson C, Snuderl M, Kirkpatrick ND, Goveia J, Davidson C, Huang Y, Riedemann L, Taylor J, Ivy P, Duda DG, Ancukiewicz M, Plotkin SR, Chi AS, Gerstner ER, Eichler AF, Dietrich J, Stemmer-Rachamimov AO, Batchelor TT and Jain RK: Increase in tumor-associated macrophages after antiangiogenic therapy is associated with poor survival among patients with recurrent glioblastoma. Neuro Oncol (2013) 15: 1079–1087.
119. van den Bent MJ, Gao Y, Kerkhof M, Kros JM, Gorlia T, van Zwieten K, Prince J, van Duinen S, Sillevis Smitt PA, Taphoorn M and French PJ: Changes in the EGFR amplification and EGFRvIII expression between paired primary and recurrent glioblastomas. Neuro Oncol (2015) 17: 935–941.
120. Cioca A, Olteanu EG, Gisca MD, Morosanu CO, Marin I and Florian IS: Expression of EGFR in Paired New and Recurrent Glioblastomas. Asian Pac J Cancer Prev (2016) 17: 4205–4208.
121. Inoue S, Ichikawa T, Kurozumi K, Maruo T, Onishi M, Yoshida K, Fujii K, Kambara H, Chiocca EA and Date I: Novel animal glioma models that separately exhibit two different invasive and angiogenic phenotypes of human glioblastomas. World Neurosurg (2012) 78: 670–682.
122. Maruo T, Ichikawa T, Kanzaki H, Inoue S, Kurozumi K, Onishi M, Yoshida K, Kambara H, Ouchida M, Shimizu K, Tamaru S, Chiocca EA and Date I: Proteomics-based analysis of invasion-related proteins in malignant gliomas. Neuropathology (2013) 33: 264–275.
123. Onishi M, Ichikawa T, Kurozumi K, Inoue S, Maruo T, Otani Y, Fujii K, Ishida J, Shimazu Y, Yoshida K, Michiue H, Antonio Chiocca E and Date I: Annexin A2 regulates angiogenesis and invasion phenotypes of malignant glioma. Brain Tumor Pathol (2015) 32: 184–194.
124. Otani Y, Ichikawa T, Kurozumi K, Inoue S, Ishida J, Oka T, Shimizu T, Tomita Y, Hattori Y, Uneda A, Matsumoto Y, Michiue H and Date I: Fibroblast growth factor 13 regulates glioma cell invasion and is important for bevacizumab-induced glioma invasion. Oncogene (2018) 37: 777–786.
125. Chen L, Lin L, Xian N and Zheng Z: Annexin A2 regulates glioma cell proliferation through the STAT3-cyclin D1 pathway. Oncol Rep (2019) 42: 399–413.
126. Kling T, Ferrarese R, D Oh, Johansson P, Heiland DH, Dai F, Vasilikos I, Weyerbrock A, Jornsten R, Carro MS and Nelander S: Integrative Modeling Reveals Annexin A2-mediated Epigenetic Control of Mesenchymal Glioblastoma. EBioMedicine (2016) 12: 72–85.
127. Li X, Nie S, Lv Z, Ma L, Song Y, Hu Z, Hu X, Liu Z, Zhou G, Dai Z, Song T, Liu J and Wang S: Overexpression of Annexin A2 promotes proliferation by forming a Glypican 1/c-Myc positive feedback loop: prognostic significance in human glioma. Cell Death Dis (2021) 12: 261.
128. Ma K, Chen X, Liu W, Yang Y, Chen S, Sun J, Ma C, Wang T and Yang J: ANXA2 is correlated with the molecular features and clinical prognosis of glioma, and acts as a potential marker of immunosuppression. Sci Rep (2021) 11: 20839.
129. Maule F, Bresolin S, Rampazzo E, Boso D, Della Puppa A, Esposito G, Porcù E, Mitola S, Lombardi G, Accordi B, Tumino M, Basso G and Persano L: Annexin 2A sustains glioblastoma cell dissemination and pro-

liferation. Oncotarget (2016) 7: 54632–54649.
130. Tatenhorst L, Rescher U, Gerke V and Paulus W: Knockdown of annexin 2 decreases migration of human glioma cells in vitro. Neuropathol Appl Neurobiol (2006) 32: 271–277.
131. Tu Y, Xie P, Du X, Fan L, Bao Z, Sun G, Zhao P, Chao H, Li C, Zeng A, Pan M and Ji J: S100A11 functions as novel oncogene in glioblastoma via S100A11/ANXA2/NF-κB positive feedback loop. J Cell Mol Med (2019) 23: 6907–6918.
132. Zhai H, Acharya S, Gravanis I, Mehmood S, Seidman RJ, Shroyer KR, Hajjar KA and Tsirka SE: Annexin A2 promotes glioma cell invasion and tumor progression. J Neurosci (2011) 31: 14346–14360.
133. Chen M, Ren R, Lin W, Xiang L, Zhao Z and Shao B: Exploring the oncostatin M (OSM) feed-forward signaling of glioblastoma via STAT3 in pan-cancer analysis. Cancer Cell Int (2021) 21: 565.
134. Guo Q, Guan GF, Cao JY, Zou CY, Zhu C, Cheng W, Xu XY, Lin ZG, Cheng P and Wu AH: Overexpression of oncostatin M receptor regulates local immune response in glioblastoma. J Cell Physiol (2019).
135. Jahani-Asl A, Yin H, Soleimani VD, Haque T, Luchman HA, Chang NC, Sincennes MC, Puram SV, Scott AM, Lorimer IA, Perkins TJ, Ligon KL, Weiss S, Rudnicki MA and Bonni A: Control of glioblastoma tumorigenesis by feed-forward cytokine signaling. Nat Neurosci (2016) 19: 798–806.
136. Natesh K, Bhosale D, Desai A, Chandrika G, Pujari R, Jagtap J, Chugh A, Ranade D and Shastry P: Oncostatin-M differentially regulates mesenchymal and proneural signature genes in gliomas via STAT3 signaling. Neoplasia (2015) 17: 225–237.
137. Repovic P, Fears CY, Gladson CL and Benveniste EN: Oncostatin-M induction of vascular endothelial growth factor expression in astroglioma cells. Oncogene (2003) 22: 8117–8124.
138. Sharanek A, Burban A, Hernandez-Corchado A, Madrigal A, Fatakdawala I, Najafabadi HS, Soleimani VD and Jahani-Asl A: Transcriptional control of brain tumor stem cells by a carbohydrate binding protein. Cell Rep (2021) 36: 109647.
139. Sharanek A, Burban A, Laaper M, Heckel E, Joyal JS, Soleimani VD and Jahani-Asl A: OSMR controls glioma stem cell respiration and confers resistance of glioblastoma to ionizing radiation. Nat Commun (2020) 11: 4116.
140. Wang S and Xu X: An Immune-Related Gene Pairs Signature for Predicting Survival in Glioblastoma. Front Oncol (2021) 11: 564960.
141. Bharadwaj A, Bydoun M, Holloway R and Waisman D: Annexin A2 heterotetramer: structure and function. Int J Mol Sci (2013) 14: 6259–6305.
142. O'Connell PA, Surette AP, Liwski RS, Svenningsson P and Waisman DM: S100A10 regulates plasminogen-dependent macrophage invasion. Blood (2010) 116: 1136–1146.
143. Phipps KD, Surette AP, O'Connell PA and Waisman DM: Plasminogen receptor S100A10 is essential for the migration of tumor-promoting macrophages into tumor sites. Cancer Res (2011) 71: 6676–6683.
144. Swisher JF, Burton N, Bacot SM, Vogel SN and Feldman GM: Annexin A2 tetramer activates human and murine macrophages through TLR4. Blood (2010) 115: 549–558.
145. Swisher JF, Khatri U and Feldman GM: Annexin A2 is a soluble mediator of macrophage activation. J Leukoc Biol (2007) 82: 1174–1184.
146. Kucia-Tran JA, Tulkki V, Scarpini CG, Smith S, Wallberg M, Paez-Ribes M, Araujo AM, Botthoff J, Feeney M, Hughes K, Caffarel MM and Coleman N: Anti-oncostatin M antibody inhibits the pro-malignant effects of oncostatin M receptor overexpression in squamous cell carcinoma. J Pathol (2018) 244: 283–295.
147. Rajpurkar P, Chen E, Banerjee O and Topol EJ: AI in health and medicine. Nature Medicine (2022).
148. Aerts HJ, Velazquez ER, Leijenaar RT, Parmar C, Grossmann P, Carvalho S, Bussink J, Monshouwer R, Haibe-Kains B, Rietveld D, Hoebers F, Rietbergen MM, Leemans CR, Dekker A, Quackenbush J, Gillies RJ and Lambin P: Decoding tumour phenotype by noninvasive imaging using a quantitative radiomics approach. Nat Commun (2014) 5: 4006.
149. Gillies RJ, Kinahan PE and Hricak H: Radiomics: Images Are More than Pictures, They Are Data. Radiology (2016) 278: 563–577.
150. Cluceru J, Interian Y, Phillips JJ, Molinaro AM, Luks TL, Alcaide-Leon P, Olson MP, Nair D, LaFontaine M, Shai A, Chunduru P, Pedoia V, Villanueva-Meyer JE, Chang SM and Lupo JM: Improving the noninvasive classification of glioma genetic subtype with deep learning and diffusion-weighted imaging. Neuro Oncol (2021).
151. Gates EDH, Lin JS, Weinberg JS, Prabhu SS, Hamilton J, Hazle JD, Fuller GN, Baladandayuthapani V, Fuentes DT and Schellingerhout D: Imaging-Based Algorithm for the Local Grading of Glioma. AJNR Am J Neuroradiol (2020) 41: 400–407.
152. Rathore S, Niazi T, Iftikhar MA and Chaddad A: Glioma Grading via Analysis of Digital Pathology Images Using Machine Learning. Cancers (2020) 12: 578.
153. Rudie JD, Rauschecker AM, Bryan RN, Davatzikos C and Mohan S: Emerging Applications of Artificial Intelligence in Neuro-Oncology. Radiology (2019) 290: 607–618.
154. Sepehri K, Song X, Proulx R, Hajra SG, Dobberthien B, Liu CC, D'Arcy RCN, Murray D and Krauze AV: Towards effective machine learning in medical imaging analysis: A novel approach and expert evaluation of high-grade glioma 'ground truth' simulation on MRI. Int J Med Inform (2021) 146: 104348.
155. Singh G, Manjila S, Sakla N, True A, Wardeh AH, Beig N, Vaysberg A, Matthews J, Prasanna P and Spektor V: Radiomics and radiogenomics in gliomas: a contemporary update. Br J Cancer (2021) 125: 641–657.
156. Zhou M, Scott J, Chaudhury B, Hall L, Goldgof D, Yeom KW, Iv M, Ou Y, Kalpathy-Cramer J, Napel S, Gillies R, Gevaert O and Gatenby R: Radiomics in Brain Tumor: Image Assessment, Quantitative Feature Descriptors, and Machine-Learning Approaches. AJNR Am J Neuroradiol (2018) 39: 208–216.
157. Zhuge Y, Ning H, Mathen P, Cheng JY, Krauze AV, Camphausen K and Miller RW: Automated glioma grading on conventional MRI images using deep convolutional neural networks. Med Phys (2020) 47: 3044–3053.
158. Bowman RL, Wang Q, Carro A, Verhaak RG and Squatrito M: GlioVis data portal for visualization and analysis of brain tumor expression datasets. Neuro Oncol (2017) 19: 139–141.
159. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstråle M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D and Groop LC: PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet (2003) 34: 267–273.
160. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES and Mesirov JP: Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A (2005) 102: 15545–15550.