REVIEW

nature neuroscience

An epigenetic gateway to brain tumor cell identity

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Precise targeting of genetic lesions alone has been insufficient to extend brain tumor patient survival. Brain cancer cells are diverse in their genetic, metabolic and microenvironmental compositions, accounting for their phenotypic heterogeneity and disparate responses to therapy. These factors converge at the level of the epigenome, representing a unified node that can be disrupted by pharmacologic inhibition. Aberrant epigenomes define many childhood and adult brain cancers, as demonstrated by widespread changes to DNA methylation patterns, redistribution of histone marks and disruption of chromatin structure. In this Review, we describe the convergence of genetic, metabolic and microenvironmental factors on mechanisms of epigenetic deregulation in brain cancer. We discuss how aberrant epigenetic pathways identified in brain tumors affect cell identity, cell state and neoplastic transformation, as well as addressing the potential to exploit these alterations as new therapeutic strategies for the treatment of brain cancer.

Brain tumors encompass a wide spectrum of over 120 histologically, demographically, clinically and molecularly distinct diseases[1] and are one of the most common causes of cancer-related death in children and adults. Genome sequencing studies have uncovered the landscape of genetic alterations present in many pediatric and adult cancer types[2], and they highlight a convergence on deregulated epigenomes in the form of aberrant DNA methylation signatures, histone modification patterns and disorganized chromatin architecture[3–7]. In adult glioblastoma (GBM, World Health Organization grade IV glioma), the most aggressive and prevalent adult primary intrinsic brain cancer, nearly 46% of patients harbor at least one mutation of an epigenetic regulator amid a diversity of oncogenic pathway mutations[8]. Equally striking is the pediatric counterpart of glioblastoma, where one highly prevalent mutation occurs in a histone protein[9]. Somatic mutations and structural variations that target regulators of epigenetic modifications and functional regulatory elements have been reported across several aggressive pediatric and adult brain cancers, such as glioblastoma[5,8–10], medulloblastoma[6,11–18], ependymoma[19], atypical teratoid rhabdoid tumors (ATRT)[20,21], diffuse intrinsic pontine gliomas (DIPG)[22–27] and embryonal tumors with multilayered rosettes (ETMR)[28]. The function of these epigenetic alterations is likely context dependent, but they ultimately influence cell identity and cell state transitions during neoplastic transformation (**Fig. 1**).

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Received 9 June; accepted 30 October; published online 29 December 2015; doi:10.1038/nn.4190

Brain cancer cells are not only heterogeneous in their genetic composition, but also reside in varying microenvironments and interact with different cell types. Therefore, factors such as altered cellular metabolism and the microenvironment may critically define the neoplastic effects of epigenetic programs in the process of brain tumor development[7,29–41]. In this Review, we will detail the collective genetic, metabolic and microenvironmental alterations present during brain tumorigenesis and discuss the impact these changes have on epigenetic programs important for cell state transition or maintenance. Further, we will highlight the therapeutic potential of targeting brain tumor cell state by modulation of epigenetic signatures.

The epigenetic gateway to cell identity and neoplastic transformation

Cancer cells are characterized by a state of uncontrolled proliferation and replicative immortality[42]. The epigenetic landscape defines cell state, supporting epigenetic control as an essential node of transformation. It is now clear, on the basis of Nobel prize–winning work of Shinya Yamanaka[43] and that of many others, that the state of a cell is dynamic and more plastic than previously thought. Various studies demonstrating direct cell conversion to specific lineages, including multiple types of neural progenitors that are the putative cell of origin of many brain tumors, highlight the ability of cells to transform their state with the introduction of only a few transcription factors[44–46]. Cancer cells capitalize on this cellular plasticity to acquire developmental programs that endow on the cell limitless self-renewal capacity, similar to that of reprogrammed induced pluripotent stem cells (iPSCs) and neural stem cells. In fact, there are close parallels between cellular reprogramming and oncogenic transformation. Yamanaka transcription factors, including SOX2 and MYC[47–49], and many of the epigenetic modifier genes that are necessary for cellular reprogramming act oncogenically (reviewed in ref. 50). Suvà *et al.*[48] demonstrated that, similarly to direct conversion of untransformed cells, they could reprogram a differentiated cancer cell into a tumor-propagating cell—satisfying a key functional criterion for glioma brain tumor stem cells (BTSCs)—with four master transcription factors: POU3F2, SOX2,

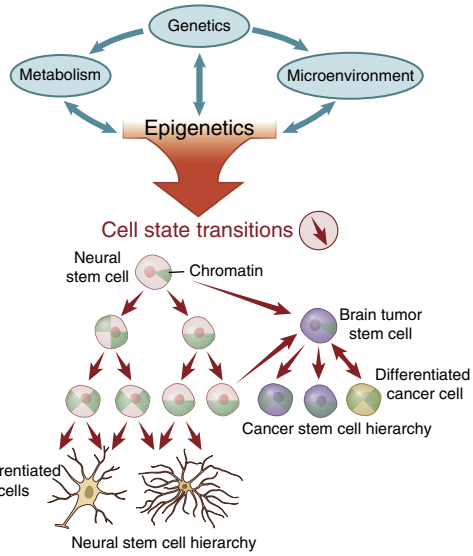

Figure 1 The epigenetic gateway to cell identity and neoplastic transformation. Top, the genetic, metabolic and microenvironmental interactions (arrows) with epigenetic programs in cancer. Bottom, the cell state transitions (red arrows) influenced by altered epigenetic landscapes and their relevance to both normal neural stem cell and cancer stem cell hierarchies. Green pie slices within the cells represent the restructuring of chromatin architecture and progression toward closed chromatin in the most differentiated cell state.

SALL2 and OLIG2 (ref. 48). Restoring, at least in part, the epigenome of a native BTSC is necessary to regain tumorigenic potential, supporting the concept that epigenomic programs define the cancer cell state. Resetting the epigenetic landscape of BTSCs, using a method similar to iPSC reprogramming, establishes an epigenetic program that is distinct from that of brain tissue and which attenuates tumor formation[51,52].

While these studies and others demonstrate in a laboratory setting that epigenetic regulation can drive or inhibit cancer growth, human tumors are not formed from the exogenous introduction of transcription factors. Tumors—in particular, brain tumors—are heterogeneous at the single-cell level and organized in a hierarchical structure composed of cells with varying cell states[53,54]. Genetic alterations, signaling alterations, metabolic alterations and microenvironmental conditions converge to dictate the epigenetic landscape of individual cells (**Fig. 1**). This landscape, in turn, defines cell state and influences cell signaling, metabolism, the microenvironment and even the genetic landscape[15,55–58]. Molecular alterations within cancer cells promote cancer growth, but multiple deregulated pathways may converge to create an oncologic epigenome: an altered epigenome that may lock cells in a stem-like state, inhibiting normal differentiation[19,53,59–61]. In concert, tumor epigenomes inhibit tumor suppressor gene expression, drive oncogenic activation and further render the cell of origin susceptible to neoplastic transformation[2,55–57,62].

Convergence on chromatin architecture

Characterization of histone modifications and their role in normal cellular function has provided insight into the potential mechanisms of epigenetic deregulation in brain cancer[63,64]. Octamers of histone proteins are responsible for wrapping 147-base-pair units of double-stranded DNA into compacted subunits called nucleosomes. Post-translational modification of histones by methylation, acetylation, phosphorylation, sumoylation, ubiquitylation and so forth instructs states of euchromatin and heterochromatin (as reviewed in ref. 65). Histone modifications further define distinct regions of the epigenome, such as enhancers, promoters and gene bodies (**Fig. 2**). Modifications of histone amino acid residues are mediated by enzymes ('epigenetic writers'), such as histone methyltransferases (for example, Enhancer of Zeste homolog 2, EZH2) and acetyltransferases (for example, P300 CREB-binding protein, CBP), that catalyze the addition of methyl or acetyl groups, and histone demethylases (for example, Jumonji domain containing 3, JMJD3) and deacetylases (for example, histone deacetylases, HDACs), which facilitate their removal ('epigenetic erasers'). Proteins that recognize histone modifications, known as histone 'readers', recruit additional proteins and protein complexes that facilitate transcriptional regulation. The organization of larger scale chromatin structure is regulated by chromatin remodelers and chromatin-associated proteins. In brain cancer, mutations have been identified at nearly all levels of chromatin regulation, from mutations of histones to enzymes that catalyze histone modification to proteins that facilitate larger order chromatin structure (**Fig. 2**).

Childhood tumors highlight epigenetic dependencies in brain cancer

An emerging theme in brain cancer sequencing studies is that fewer mutations are observed in childhood brain tumors than in adult[5,6,8,16,17,19,20,66–71]. This holds true for other pediatric cancers, such as infant leukemia, neuroblastoma and retinoblastoma, which exhibit lower mutation rates as compared to highly mutated adult tumors, such as melanoma and lung cancer[17,19,66–68,70,71]. Of the few recurrent mutations identified in brain cancer genomes, many target chromatin-associated proteins or histone proteins themselves. The genes encoding these are termed 'landscaping genes' owing to their potential widespread effects on transcriptional programs[4–9,12,13,15,17,19,28,69]. ATRTs harbor remarkably silent genomes, yet exhibit recurrent mutations or deletions of the *SMARCB1* gene (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily B)[20,21,72] (**Fig. 2**). *SMARCB1* encodes a subunit of the SWI/SNF chromatin-remodeling complex that functions as a tumor suppressor protein and is highly mutated in several cancers[73]. Homozygous deletion of *SMARCB1* in mice leads to embryonic lethality, while heterozygous loss leads to aggressive tumors that recapitulate human rhabdoid tumors[74–76]. It is important to note that *SMARCB1* loss is deleterious to a vast majority of cells, and mutation in an exclusive cellular and developmental context leads to neoplastic transformation[77]. As shown in *Drosophila* neuroblasts, proper lineage specification by the SWI/SNF component Osa (ARID1) prevents tumorigenesis by restricting self-renewal and inhibiting dedifferentiation[78]. Two groups recently described the genetic landscape of another aggressive pediatric brain tumor, ependymoma, in which hindbrain tumors exhibit no recurrent mutations in coding space and no evidence of recurrent gene fusions or focal somatic copy number alterations[19,79,80]. This was in contrast to its direct adult ependymoma counterpart, which harbors widespread genomic instability[81]. The DNA methylome of infant hindbrain ependymoma displays aberrant DNA hypermethylation at CpG islands described as a CpG island methylator phenotype (CIMP). Notably, hypermethylated genes converge on embryonic stem cell (ESC) targets regulated by the Polycomb repressor complex 2 (PRC2), suggesting that epigenomic alterations could be disrupting cell state and differentiation programs important to ependymoma development. A link between ESC programs and cancer is further demonstrated in the embryonal brain tumor ETMR, which harbors a fusion between a highly amplified microRNA cluster (C19MC) and *TTYH1* (Tweety family member 1)[28,82,83]. A downstream consequence of the fusion is aberrant overexpression

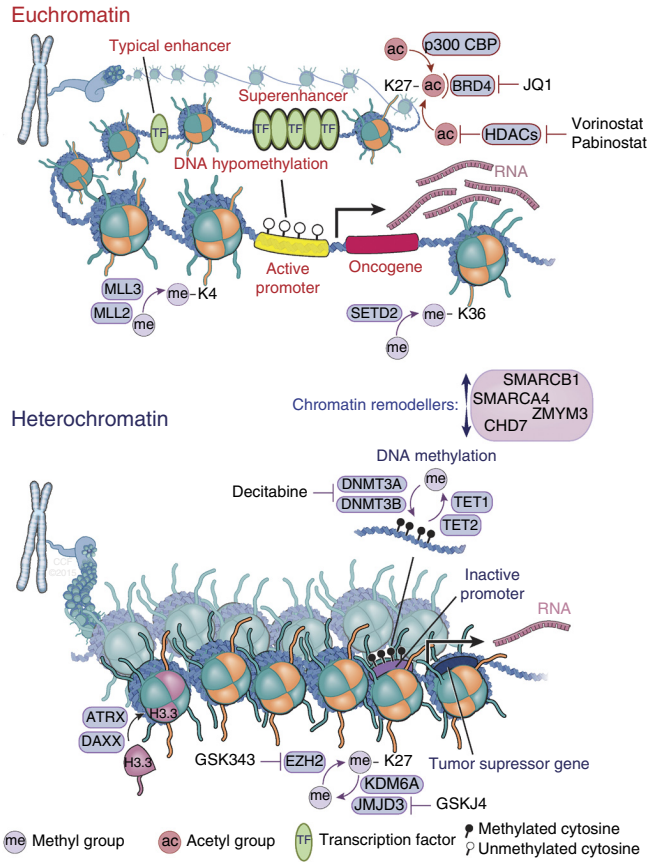

Figure 2 Brain tumors converge on chromatin architecture. Top, euchromatin and histone modifications that mediate active transcription in cancer cells. Shown are various histone modifications and enzymes, which catalyze the addition of post-translational modifications, such as histone methylation and acetylation, or which bind to these modifications, such as BRD4, which binds acetylated lysine residues on histones. The green ovals represent transcription factor binding sites and locations of enhancers or clusters of enhancers, termed superenhancers. Also shown are drug compounds that inhibit the removal (vorinostat and pabinostat, histone deacetylase (HDAC) inhibitors) or detection (JQ1) of acetylation. Center, chromatin remodelers, which facilitate the landscape of higher order chromatin structure toward euchromatin or heterochromatin. Bottom, heterochromatin and the associated modifications that mediate tumor suppressor gene silencing. These include the DNA methyltransferase family of enzymes, which catalyze the addition of methyl groups to cytosine-guanine dinucleotides, and TET enzymes, which facilitate DNA demethylation through 5-methylcytosine hydroxylation. Also shown is EZH2, which methylates histone H3 at position 27, and the associated histone H3K27 demethylases KDM6A and JMJD3. Chemical inhibitors reverse the methylation marks deposited or removed by these methyltransferase and demethylase enzymes related to heterochromatin (decitabine, GSK343, GSKJ4). ATRX and DAXX function to incorporate the histone H3.3 variant and are frequently mutated in pediatric high-grade glioma.

of a DNA methyltransferase 3B (*DNMT3B*) isoform normally expressed exclusively in the first weeks of neural tube development. Observations in ATRT, ependymoma and ETMR, along with several other cancers, suggest that neoplastic transformation is a process dependent on proper maintenance of stem cell programs through tight chromatin regulation. While these aberrant epigenetic events have been observed through genome-wide approaches, future validation will be needed to model these alterations during the initiation and progression processes of brain tumorigenesis.

Mutations of histone proteins

Recurrent genetic lesions linking epigenomic programs to brain tumor formation is perhaps best exemplified in pediatric glioblastoma and DIPG, which harbor frequent mutation of *H3F3A*, encoding the H3.3 histone variant, and to a lesser extent *HISTH1B* and *HISTH1C*, encoding the H3.1 variant[9,22,23,26,84–87]. These mutations target histone H3 lysine 27 (H3K27), an important site of direct epigenetic post-translational modifications, with a K27M mutation, or introduce a G34R or G34V mutation, which is thought to affect a nearby lysine residue at position 36 (H3K36)[10] (**Fig. 2**). The H3.3 K27M mutation is associated with globally decreased K27 methylation (K27me) and increased K27 acetylation (K27ac)[88]. Further, the K27M mutant results in aberrant redistribution of residual patterns of H3K27 trimethylation (H3K27me3) in the tumor epigenome[85,86]. ESC-derived neural precursor cells (NPCs) can be transformed with a combination of H3.3-K27M overexpression, short hairpin RNA knockdown of TP53 and overexpression of PDGFRA (platelet-derived growth factor receptor A)[89]. Notably, ESCs and terminally differentiated cells are resistant to transformation, suggesting that the effect of the K27M mutation is restricted to a cell type occurring within a defined NPC population during embryonic development. The temporally and anatomically distinct tumors defined by K27M and G34R or G34V mutations suggest unique cells of origin and/or cell states that are required for tumor initiation[87]. Mutations have also been reported in the proteins that facilitate histone H3.3 incorporation, such as α-thalassemia/mental retardation syndrome X-linked (ATRX) and death-domain associated protein (DAXX)[9,90]. The significance and functional characterization of these mutations in the setting of epigenomic reprogramming remains an area of active and future investigation.

Mutations of histone modifiers

Enzymes that catalyze the addition or removal of modifications are recurrently mutated, amplified or deleted in brain cancer genomes. These include mutations in *MLL2* and *MLL3* (mixed-lineage leukemia 2 and 3, respectively; in medulloblastoma and adult glioblastoma)[6,16,17,69], *SMARCB1* (in ATRT)[20,21], *SMARCA4* (in glioblastoma, medulloblastoma, ATRT)[5,8,9,14,16,17,69,87,91] and *SETD2* (SET domain containing 2; in both pediatric and adult glioblastoma)[91] occurring in a diverse set of adult and pediatric brain tumors (**Fig. 2**). Whole-exome and whole-genome sequencing studies of medulloblastoma have revealed the most commonly mutated chromatin modifier to be *MLL2*, which mediates histone H3 lysine 4 (H3K4me3) trimethylation, a mark of active transcription[6,11,14,16,17]. Further, the histone K27 demethylase *KDM6A* is recurrently mutated and is associated with increased H3K27me3 levels in a group of medulloblastomas with a poor prognosis (group 4), which also overexpress EZH2. Poor prognosis medulloblastomas (groups 3 and 4, which are not driven by sonic hedgehog (SHH) and Wnt signaling) also harbor subgroup-associated mutations in *CHD7* (chromodomain helicase DNA binding protein 7) and *ZMYM3* (zinc finger, MYM-type 3), which converge on regulation of gene expression by H3K4me3. Given the role of H3K27me3 in repressing lineage-specific genes in stem cells, it is hypothesized that group 3 and 4 medulloblastomas retain stem-like signatures through accumulation of H3K27me3 and abrogation of H3K4me3-mediated transcription. Notably, these alterations are in contrast to the global loss of H3K27me3 levels in pediatric glioblastoma and perhaps suggest that perturbation of a global balance and/or distribution of H3K27me3 and H3K4me3 patterns may reflect cell-state-specific dependencies in neoplastic transformation. A major future effort will be functional characterization of these epigenetic

alterations and identification of specific developmental cell types where their epigenetic deregulation promotes tumor formation.

Genomic regulatory elements of brain tumors

The convergence on histone modifications and chromatin regulation highlights the importance of understanding and mapping these modifications in brain tumors. In tumors such as pediatric glioblastoma and ependymoma, histone modification mapping by chromatin immunoprecipitation followed by high density sequencing (ChIP-seq) has demonstrated aberrant epigenetic patterns of histone H3K27 trimethylation[10,19,85]. The linkage between epigenetic modifications and cell identity and lineage specification underscores the importance of understanding the epigenetic landscape in brain cancer. Recent studies have highlighted the importance of clusters of enhancer elements, termed superenhancers, that both identify and regulate genes involved in cell identity and disease[92] (**Fig. 2**). These epigenomic features can be co-opted in cancer by mutations and structural variations[93]. In group 3 medulloblastoma, superenhancers are hijacked by structural variations, which lead to aberrant activation of *GFI1* and *GFI1b* (growth factor independent 1 transcription repressor) oncogenes[12]. In several brain tumors, noncoding mutations have been observed in the promoter regions of *TERT* (telomerase reverse transcriptase, which encodes the catalytic subunit of the enzyme telomerase), which are enriched in tumors characterized by low rates of self renewal[94,95]. The consequence of these mutations in glioblastoma is the aberrant recruitment of the GABP (GA binding protein) transcription factor[96]. Future in-depth sequencing of noncoding regions and integration with histone modification and transcription factor maps may uncover crucial genes that maintain cell state and the factors that govern their expression.

Altered DNA methylation patterns in brain cancer

Changes in DNA methylation patterns have been widely reported in cancer in the form of DNA hypermethylation and silencing of tumor suppressor genes, and loss of methylation of oncogenes and repetitive elements[97]. So far, genome-wide studies focusing largely on promoter regions and CpG islands have revealed new mechanisms of oncogenic and tumor suppressor gene regulation in cancer. Examples include widespread accumulation of DNA methylation in *IDH1* (isocitrate dehydrogenase 1)-mutated gliomas (see below)[39,98] and the establishment of CIMP phenotypes in other tumors, such as ependymoma (**Fig. 2**). Further, an important application of DNA methylation profiling is to identify signatures associated with genetic lesions and to use DNA methylation as a method for robust molecular stratification[8,19,21,87]. It is also posited that DNA methylation patterns may reflect the specific cellular states and/or cells of origin present during transformation. Advances in our understanding of the epigenomic landscapes of normal human and murine neural stem cells and cellular hierarchies may shed light on the potential cell identity and cell state transitions that occur in the early stages of brain tumor initiation. Technological advances have also allowed genome-wide characterization of brain tumor DNA methylomes using whole-genome bisulfite sequencing. Early whole-genome bisulfite sequencing studies have revealed new mechanisms of transcriptional regulation in medulloblastoma and ependymoma, and have provided an integrated view of DNA methylation and histone modification landscapes in brain tumors[15,19].

Epigenetic perturbation of genetic landscapes

In addition to influences on cell state, epigenetic alterations have been shown to have widespread effects on the genetic landscape of tumor cells. For example, methylated cytosine bases are highly prone to mutation by spontaneous deamination to thymine, thus creating opportunities for deregulation of tumor suppressor genes and oncogenes in the absence of intact DNA repair mechanisms[99]. Furthermore, hypomethylation of transposable elements have been observed widely in cancer and may contribute to genomic instability through aberrant translocation of DNA sequences[100]. At the chromatin level, a direct association between histone modifications and genetic alteration is evidenced in tumors that overexpress the H3K9me3 and H3K36me3 lysine demethylase KDM4A, also known as JMJD2A, which leads to regional DNA copy gain in the absence of global chromosomal instability[58]. This illustrates a scenario in which aberrant chromatin modulator expression could establish somatic copy number changes during neoplastic transformation[58]. From cancer genome sequencing studies, evidence is emerging linking regional mutation density to the degree of heterochromatin as marked by H3K9me3 (ref. 56). These findings demonstrate that somatic mutations are not distributed uniformly across the human genome and that they are associated with epigenomic topographies derived from the most likely cell type of origin and cell state during malignant transformation[55].

Cellular microenvironment influences epigenetic state of brain tumor cells

Brain tumor cells do not exist in isolation, but are part of a dynamic and spatially distributed system, interacting with a wide diversity of environments and cell types. For example, active neuronal activity promotes mitosis of the putative cells of origin in high-grade glioma through NLGN3 (neuroligin 3) secretion[101]. BTSCs, in particular, exhibit a complex relationship with their microenvironment: they can actively modify and shape their own environment but are also regulated, supported and directed by microenvironmental signals (**Fig. 3**). This intricate crosstalk is crucial to maintaining a stem cell state and occurs in a localized, supportive microenvironment around the stem cells called a niche. There are a multitude of factors in the stem cell niche that affect the cellular state of brain tumor cells. These include nutrient availability, hypoxia, pH and cell-cell interactions. In other systems, stem cell state maintenance and cell state change or differentiation are governed epigenetically[102]. So far, little is known at the mechanistic level as to how niche cues regulate brain tumor epigenetics. However, a number of studies have revealed how external environmental cues functionally change brain tumor cell state through unexplored epigenetic mechanisms.

The hypoxic niche. Areas of hypoxia and necrosis can be a diagnostic feature of many malignant tumors, including glioblastoma. Historically, this has been hypothesized as the expected occurrence when a tumor's growth outpaces its blood supply, leaving behind starved and/or dying cells, but recent studies have revealed that micro- and macrocellular relationships within a tumor's hypoxic niche are far more complex. Many normal adult stem cell niches, as well many steps of embryonic development, are naturally hypoxic[103]. Hypoxic niche support of stem cells may be a feature conserved among development, normal tissue maintenance and cancer. Although cells in nutrient-rich environments have the resources to facilitate rapid proliferation and tumor growth, it may be the cells in the hypoxic niche that actually drive tumor progression and recurrence owing to the stem-like transcriptional and epigenetic adaptations they undergo in this environment (**Fig. 3**).

The direct molecular responses of brain tumor cells to hypoxia are principally mediated by the hypoxia-inducible factor (HIF) family of transcription factors, especially HIF1α and HIF2α[104]. In glioblastoma biopsies, BTSCs are enriched in perinecrotic regions in the context of

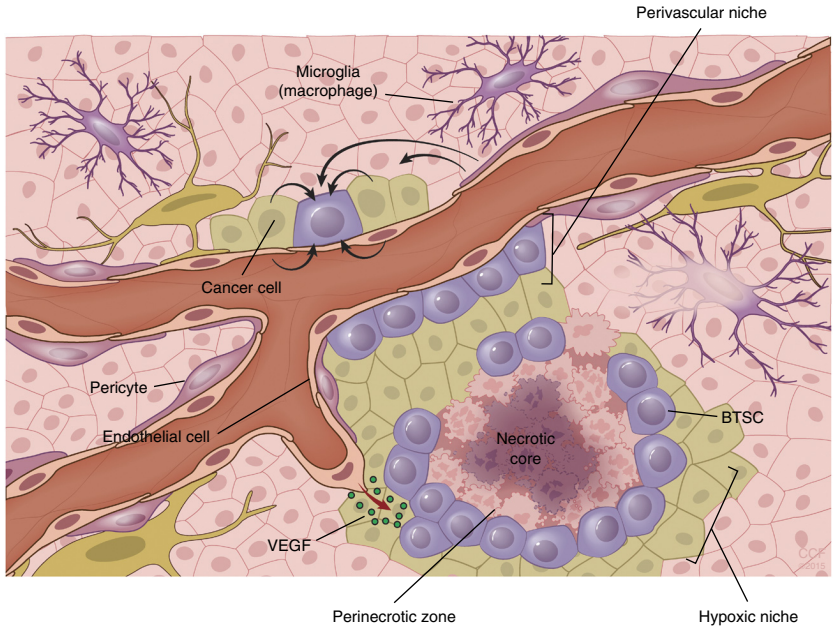

Figure 3 Cellular microenvironment influences epigenetic state of brain tumor cells. The brain tumor microenvironment includes both perivascular and hypoxic niches, which dictate interacting cell types and nutrient availabilities. Both cancer cells (light green) and brain tumor cells (round violet) exist in dynamic microenvironments containing exogenous signals from surrounding microglia (purple), pericytes (dark pink), endothelial cells (light pink) and other neoplastic cells. These interactions occur in the presence of variable growth factor (for example, VEGF) gradients, oxygen availability and nutrient levels (glucose, acetate, glutamine and so forth).

HIF activation[105]. A number of studies have demonstrated that hypoxia directly mediates expansion of the BTSC pool and that this is dependent on HIF1 and HIF2 (refs. 31,106). However, whereas HIF1α appears generally necessary for glioma survival in hypoxia, HIF2α is specifically necessary to sustain BTSCs[31]. This may be mediated through HIF2 enhancement of MYC transcriptional activity[30], which is required for BTSC maintenance and proliferation[107].

Little is known about the direct epigenetic consequences of hypoxia and HIF activation in brain cancer, but exploration is beginning. In NPCs of the developing brain, HIF1α interacts with Notch signaling and can affect cell fate decisions through epigenetic alteration[41]. In glioblastoma, the histone methyltransferase MLL1 (mixed-lineage leukemia 1) is induced by hypoxia; and loss of MLL1 reduces the expression of HIF transcripts and HIF2α protein[108], indicating a potential feedback loop sustaining the hypoxic response. Depletion of MLL1 inhibits the expression of HIF2α and target genes, including vascular endothelial growth factor (VEGF), and reduces BTSC self-renewal, growth and tumorigenicity[108].

In other cancers, HIF-independent hypoxia-mediated epigenetic silencing of tumor suppressor genes has been described. Specifically, the *BRCA1* and *RAD51* promoters have been shown to be repressed by local chromatin restructuring via H3K4 demethylation, H3K9 methylation and H3K9 deacetylation[109]. It is important to note that a growing number of epigenetic modifiers, which are deregulated in many cancer types, are dependent on proper oxygen maintenance (see below). As one example, various cancer cell lines grown *in vitro* rather than in the hypoxic conditions they experience in an *in vivo* xenograft setting experience a global induction of DNA hypomethylation[110].

For a wide variety of cancers, extracellular solid tumor pH has been determined to be significantly more acidic than in normal tissues[111]. Tumor hypoxia in particular can induce a metabolic shift that causes acidosis[112], although these two microenvironmental components can also occur independently[32]. Notably, acidic conditions promote the expression of BTSC markers, self-renewal and tumor growth through augmentation of HIF2 transcriptional responses[35]. In response to an acidic environment and decreasing intracellular pH, cancer cells have been shown to respond with an attempt to regulate intracellular pH by global deacetylation, which is accompanied by extensive redistribution of acetylation across the genome[113]. This suggests that exposure to low pH, either derived extrinsically from the niche or created autonomously by cellular alteration of the niche, promotes malignancy through the induction of distinct cellular phenotypes (that is, the BTSC) and is a process tightly associated with epigenetic alterations.

The perivascular niche. A hallmark of glioblastoma is the development of histologic regions of microvascular proliferation, often displaying highly disorganized angiogenic vessels and overall high vascularity (**Fig. 3**). Angiogenesis is essential for tumor survival and is the canonical downstream effect of HIF transcriptional activity. Medulloblastoma cells and BTSCs both consistently secrete elevated amounts of VEGF[40,114]. This effect is markedly enhanced by hypoxia and serves to increase endothelial migration, motility and vasculogenesis[114]. This suggests initial epigenetic state changes within endothelial cells as BTSCs recruit blood vessels through VEGF secretion, followed by epigenetic adaptation of the BTSCs as they adopt a new cell state to complement their changing niche. The regions around these blood vessels are high in oxygen and nutrients and harbor an increased number of stem cells[115]. Cells in glioblastomas, medulloblastomas, ependymomas and oligodendrogliomas are located near tumor capillaries. In this perivascular niche, soluble factors released from the endothelial cells can promote self-renewal and proliferation of BTSCs[29]. In medulloblastoma, perivascular stem cells are resistant to radiation and likely give rise to tumor recurrence[116]; this echoes similar findings in glioblastoma[117].

Infiltration and enrichment of tumor-associated macrophages (TAMs) is a common feature of glioblastoma, where TAMs are preferentially located in the perivascular niche (**Fig. 3**)[118]. Their mutual enrichment and proximity has suggested a relationship between TAMs and BTSCs. Although activated M2 TAMs have well known protumor effects[119], including such an effect in glioma[120], the mechanisms of the potential BTSC-TAM relationship have been largely undefined. Recently, Zhou *et al.*[121] demonstrated that BTSCs preferentially secrete the cytokine periostin (POSTN), which attracts TAMs. POSTN repression resulted in a striking reduction in TAM density, inhibition of tumor growth and improved survival of tumor-bearing mice[121]. TAMs or microglia in the glioblastoma microenvironment may also promote TGF-β- and NF-κB-dependent mesenchymal differentiation, enabling glioblastoma cells to switch subtypes to a more radiation-resistant cell state[122]. This may further be governed through aberrant activation of the STAT3 (signal transducer and activator of transcription 3) pathway in glioma by frequent loss or repression of the tumor suppressor phosphatase PTPRD (protein tyrosine phosphatase, receptor type, D)[123].

For these state-change events to be lasting and maintained by the niche, BTSCs must adopt a stem-like chromatin state.

Epigenetic regulation of tumors by endothelial cell signals. Beyond being a good place for a stem cell to grow because of the abundance of oxygen, nutrients and growth factors, the perivascular niche contains cells that directly interact and bidirectionally communicate with BTSCs (**Fig. 3**). The molecular mechanisms through which the perivascular niche controls BTSC state are beginning to be discovered. BTSCs express Notch receptors while endothelial cells of the niche express Notch-activating ligands[124]. Whereas co-xenograft of brain tumor cells and endothelial cells increases tumor initiation and growth, knockdown of Notch ligands in the co-injected endothelial cells reduces tumor growth[29,124]. BTSCs in the hypoxic niche secrete VEGF[114], which in turn can both recruit new blood vessel formation and stimulate endothelial cells to secrete Notch ligand[125], which can then stimulate Notch signaling in BTSCs. This feedforward loop may be yet another example of microenvironmental modification initiated by BTSCs to promote maintenance of their own cell state.

Cancer cell dormancy is a potential mechanism to explain many detrimental clinical findings, including resistance to chemotherapy, tumor recurrence and metastasis[126]. Entry and exit from cancer dormancy is mediated by epigenetic alterations, signaling pathways and transcriptional circuits that are also known to drive stem cell reprogramming and maintenance[126]. The key coagulation mediator F3 (tissue factor 3), expressed by vascular endothelial cells, is linked with breast cancer progression[127], where protein secretion by endothelial cells during neovascularization may trigger an exit from dormancy and cancer proliferation[128]. In glioma, cancer cell dormancy may be governed by F3. F3 activity enables glioma cells to form a microenvironment containing angiogenic and inflammatory cells. Strikingly, glioma cells lacking F3 remain viable but dormant unless they are supplemented with exogenous F3 (ref. 129). This result suggests that microenvironmental changes triggering exit from dormancy are accompanied by more permanent epigenetic, genetic and phenotypic changes in the glioma cells resulting in tumorigenesis.

Influences from the microenvironment can affect, promote, preserve and even dictate brain tumor cell states. These findings could have vast clinical implications and suggest therapeutic targets greatly needed in this disease. However, we as yet lack the basic mechanistic understandings of how these phenotypic changes in brain tumor cell states are affected and maintained at the chromatin level. Advancing technologies that allow epigenetic analysis at high fidelity with lower numbers of cells may enable such studies to be performed in the near future.

Cellular metabolism influences brain cancer epigenetic state

The metabolic state of brain tumor cells is highly influenced by alterations in tumor microenvironment and is linked directly to changes in global epigenetic patterns (**Fig. 4**). Microenvironmental alterations dictate fuel sources available to brain tumor cells, such as glucose[130,131], acetate[33,132] and glutamine[133], which limit or alter the distribution of substrates required for post-translational epigenetic modifications[33,132]. Mutations of metabolic pathways have been observed in several cancers, in addition to brain tumors, as a means of disrupting epigenetic and cellular state[134,135]. In glioma, one of the most common recurrent mutations occurs in *IDH1*, resulting in the accumulation of an oncometabolite, (*R*)-2-hydroxyglutarate, that functions to inhibit the activity of multiple α-ketoglutarate (α-KG)-dependent dioxygenases. Through competitive inhibition, (*R*)-2-hydroxyglutarate impairs the activity a wide variety of histone and DNA demethylases, such as the JMJC domain-containing histone demethylases (KDMs), RNA demethylases and the TET (ten-eleven translocation) family of DNA hydroxylases that facilitate DNA demethylation (**Fig. 4**). These enzymes comprise a family of 2-oxoglutarate-dependent dioxygenases that depend on iron and oxygen for their function, further linking metabolic and hypoxic regulation with epigenetic programs. However, these widespread effects also increase the difficulty of deciphering the functional consequence(s) of the IDH mutations—specifically, whether some of these effects are a mere product of increased (*R*)-2-hydroxyglutarate production. One of several consequences from IDH mutations is aberrant methylation of histones at several lysine residues and acquisition of a CpG island methylator phenotype through DNA hypermethylation[37–39,98]. While the function of *IDH1* mutations in glioblastoma remains to be fully characterized, the result of increased histone methylation prevents lineage-specific progenitors from differentiating into terminally differentiated cells[38]. Furthermore, chemical inhibition of IDH1 has been shown to promote glioma differentiation[136]. Like pediatric glioblastomas, which harbor K27M mutations, the convergence on epigenetic programs elicited by metabolic state changes suggests that these types of mutations may function to activate stem or progenitor cell states required for tumorigenesis.

Like all cancers, brain cancers display the Warburg effect, a preferential utilization of aerobic glycolysis for energy supplies and macromolecule synthesis. This is especially true in the hypoxic niche, where both oxygen and nutrients supplied by distant blood vessels are scarce (**Fig. 3**). One method used by BTSCs to meet their metabolic needs is to co-opt expression of the high affinity glucose transporter, GLUT3, to efficiently scavenge glucose from their environment[137] (**Figs. 3** and **4**). More strikingly, non-stem glioblastoma cells grown in

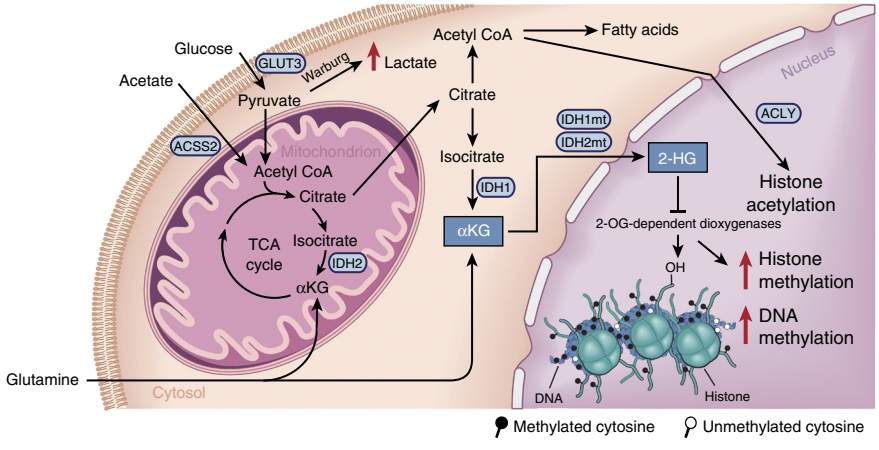

Figure 4 Cellular metabolism influences brain cancer epigenetic state. Metabolic pathways present in a brain tumor cell, with emphasis on transport proteins (GLUT3) and enzymatic effectors—IDH1 and IDH2 mutations (IDH1mt, IDH2mt), ACSS2 and ATP citrate lyase (ACLY)—which alter tumor metabolism and ultimately epigenetic programs. IDH1 mutation results in the accumulation of 2-hydroxyglutarate (2-HG), a metabolite that inhibits the function of iron, oxygen and α-ketoglutarate (αKG)-dependent demethylase enzymes, thus leading to aberrant accumulation of both DNA methylation and histone methylation. TCA, tricarboxylic acid; 2-OG, 2-oxoglutarate.

restricted glucose exhibit increased levels of the ESC master transcription factors and show functional enrichment for stem-like cells, indicating adaptation and reprogramming to a more stem-like state[137]. The exact epigenetic mechanisms underlying these adaptations are so far unknown. However, the genomic locus of *GLUT3* is part of a conserved, 200-kb gene cluster that is highly enriched for genes associated with pluripotency, including the master ESC transcription factor *NANOG*[138]. This region is under control of another the master ESC transcription factor, OCT4 (octamer-binding transcription factor 4)[139]. It is possible that cancer cells can gain GLUT3 expression and stem cell properties simultaneously by epigenetically de-repressing this region of chromatin during stem cell reprogramming.

Another mechanism used by brain tumors and brain metastases to meet tumor metabolic demands is the utilization of acetate by the enzyme ACSS2 (acetyl-coenzyme A synthetase, cytoplasmic)[33,132]. Acetate and coenzyme A are oxidized by ACSS2 to form the central metabolite acetyl-CoA, necessary for a wide variety of cellular processes, including epigenetic modulation through histone acetylation[140]. Histone acetylation has a very short half-life in tumor cells, creating an abundant supply of intracellular acetate to be used by ACSS2 (ref. 33) and also necessitating a continued active upkeep of histone modifications to maintain cell state. Indeed, acetate is used by ACSS2 in both brain tumor models and brain tumor patients, and its expression correlates with tumor aggressiveness in a variety of cancers, including brain tumors[132].

Brain tumor therapy by disruption of epigenetic regulators

The convergence of genetic, metabolic and microenvironmental alterations on cell state and the dependence of cell state on epigenomic programs suggest that targeting epigenetic mechanisms could be a valuable strategy for treatment of brain tumors. Numerous preclinical studies have shown that brain tumors are sensitive to a variety of inhibitors of epigenetic modifications, several of which are approved for use in patients by the US Food and Drug Administration (**Fig. 2**)[141–143]. These include DNA methylation inhibitors such as decitabine and HDAC inhibitors such as vorinostat. Targeted epigenetic modulation has already shown promise in numerous preclinical models of brain tumors characterized by aberrant epigenetic programs. In the case of DIPGs that harbor the H3.3 K27M mutant and show global loss of H3K27 trimethylation, an inhibitor (GSK-J4) of the H3K27 demethylase JMJD3 has been shown to be effective at reducing tumor growth by elevating H3K27 trimethylation[144]. Furthermore, GSK-J4 exhibits synergistic activity with the HDAC inhibitor pabinostat[145]. In brain tumors such as glioblastoma, ATRT and ependymoma, characterized by aberrant H3K27me3 patterns, highly specific EZH2 inhibitors (namely, GSK343) have been shown to be effective at restricting tumor growth in preclinical models[19,146,147].

A novel avenue of targeting histone modification is inhibiting the readers of acetylation (for example, BRD4, bromodomain containing 4), which mark active enhancers and superenhancers, using inhibitors of bromodomain containing proteins, such as JQ1 (ref. 148). JQ1 treatment has been shown to be effective in both MYC- and SHH-driven medulloblastoma by targeting cancer dependency genes driven by superenhancers[149,150]. These early studies represent an emerging concept: reversing epigenetic signatures in brain tumors using small molecule epigenetic inhibitors. Understanding the function and potential requirements of specific epigenetic marks in brain tumors, alongside development of specific epigenetic drugs, may reveal new opportunities for rational and targeted therapeutics. Targeting cellular state through manipulation of epigenetic regulators represents an alternative or complementary approach to drugs that target specific genetic lesions.

Moving forward

Genomic sequencing of several types of brain tumors—astrocytomas, oligodendrogliomas, medulloblastomas, ependymomas, meningiomas, ATRT—have yielded remarkably granular genomic landscapes. Glioblastoma and other brain tumors harbor mutations that are infrequent in isolation but disrupt normal function of a limited cohort of pathways (TP53, retinoblastoma, receptor tyrosine kinase signaling and chromatin-associated molecules). Sadly, this avalanche of information has had a relatively modest impact on the clinical practice of neuro-oncology. Therapeutic trials against driving genetic abnormalities amenable to therapeutic targeting, such as the epidermal growth factor receptor, have been largely negative in most brain cancers. The standard of care for most brain tumors remains focused on maximal surgical resection, radiotherapy and chemotherapy. Indirect targeting of the tumor through anti-angiogenics (for example, bevacizumab) and immunotherapies (vaccines, adoptive therapies, immune checkpoint inhibitors and oncolytic viruses) have demonstrated preclinical activity but mixed efficacy in clinical trials. The convergence of genomic alterations, microenvironmental conditions and metabolic reprogramming to create an epigenetic landscape that promotes aberrant activation and maintenance of stem cell–like transcriptional programs may offer a coherent strategy for improving diagnosis, prediction of prognosis and therapies. Global chromatin reprogramming may be detectable at the earliest stage of transformation, empowering early detection and prognosis. Circulating DNA and tumor cells have proven informative of tumor development and progression, suggesting that simultaneous assessment of tumor genetics and epigenetics may better inform concerning the status of tumors. Currently unclear is the landscape, prevalence and importance of noncoding mutations and structural variations, which will be revealed as whole genomes from brain tumors are sequenced to greater depth. Delineating the functional consequences of noncoding alterations will benefit from comprehensive and integrated mapping of histone modifications and chromatin structure in brain tumors. Epigenomic mapping, such as enhancer profiles, may also reveal the master transcription factors important for maintaining cancer cell state, in addition to the mechanisms that lead to oncogenic transformation. The many influences on epigenetic mechanisms, including both intrinsic factors (that is, mutations) and extrinsic factors (that is, microenvironment), may complicate epigenomic mapping of brain tumors. However, identifying pathways of convergence and dependencies on epigenetic programs may provide important insights into the molecular biology of brain tumors and new avenues for cell state therapies. While targeting epigenetic regulators in tumor cells—for example, inhibitors of IDH1 or BRD4—may offer benefit, sustained tumor control will be most likely achieved with combinatorial targeting strategies with conventional or targeted therapies. Potentially, inhibitors of chromatin-associated proteins could induce synthetic lethality with other treatments, disrupt the growth of heterogeneous tumor populations and attenuate mechanisms of progression. Transforming neuro-oncology care will require more complex modeling of tumor biology through the integration of epigenetics and the multidimensional interactions with genetics, metabolism and the microenvironment. We must exercise caution, as each new advance in oncology has been hailed as a potential cure, but reprogramming tumor cells toward a differentiated phenotype could reverse therapeutic resistance, immune escape, invasion and angiogenesis.

ACKNOWLEDGMENTS

We thank D. Schumick (Center of Medical Art and Photography, Cleveland Clinic) for assistance with figure preparation. This work was supported by The Banting Fellowship (S.C.M.), James S. McDonnell Foundation (J.N.R.) and US National

Institutes of Health grants: F32 CA189647 (C.G.H.), F30 CA183510 (T.E.M.), T32 GM007250 MSTP (T.E.M.), R35 CA197718 (J.N.R.), CA154130 (J.N.R.), R01 CA169117 (J.N.R.), R01 CA171652 (J.N.R.), R01 NS087913 (J.N.R.) and R01 NS089272 (J.N.R.). M.D.T. is supported by a Canadian Institutes of Health Research Clinician Scientist Phase II award, funds from the Garron Family Chair in Childhood Cancer Research at The Hospital for Sick Children and The University of Toronto, and operating funds from the Canadian Institutes of Health Research, the US National Institutes of Health (R01CA159859 and R01CA148699) and the Pediatric Brain Tumor Foundation.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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