Brain Cancer Stem Cells: The Emerging Role of Chromatin in Glioma Biology

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Abstract

Glioma encompasses a heterogeneous group of primary brain tumors of astroglial/neural stem cell origin. In most cases, these tumors can be isolated and maintained in neural stem cell culture conditions where they behave alike neural stem cells and express neural stem cell markers. In adults, glioblastoma multiforme (GBM) represents the most common and deadly brain tumor and can be sub-classify in 4 types based on marker expression and cell phenotype. Though to be highly distinct from adult GBM based on the unique genetic mutation profile and resistance to temozolomide treatment, pediatric glioma may be much closer to GBM when analyzed at the chromatin level. Recent progress in genetics and chromatin biology suggests that a common finality links adult and pediatric glioma. Herein, we will discuss the implication of these findings for the development of new therapies against these deadly tumors.

Key words: Glioblastoma, Glioma, Chromatin, Pediatric, Cancer stem cell

1. Glioma Classification

Glioma represents a heterogeneous set of diseases, ranging from benign to malignant tumors. Of which, glioblastoma multiforme (GBM), a grade IV astrocytoma (WHO), represents the most common and lethal brain malignancy in adults, with a median lifespan of 9 to 15 months at time of diagnosis for primary GBM. However, current treatments regimen are palliative and can only increase lifespan by 3-4 months. Treatments involve surgical resection, ionizing radiation and chemotherapy. An effective treatment is thus needed [1-3].

Classification of glioma in the 2007 WHO data is based on histological analysis, and tumors are graded according to the level of tumors malignancy features, wich includes necrosis, vascular proliferation, anaplasia and mitotic index [4]. However, this classification system remains limited by the heterogeneous features of gliomas, where it failed to distinguish the clinicopathologic stratification of primary GBM, which develops *de novo*, from secondary GBM, which develops from lower grade diffuse glioma [5, 6]. Recently, the 2016 WHO classification of CNS tumors uses, for the first time, molecular features in parallel to histology characteristics to define glioma entities. In the 2016 CNS WHO classification, glioblastoma are divided into: glioblastoma with IDH-wildtype (about 90% of cases), which is associated closely with the primary GBM, and glioblastoma with IDH-mutant (about 10% of cases), which is associated most frequently with secondary GBM (Table-1).

Table 1. Characteristics of IDH-wildtype and IDH-mutant glioblastoma*

Glioblastoma	IDH-wildtype	IDH-mutant
Synonym	Primary glioblastoma	Secondary glioblastoma
Origin	*de novo*	Lower grade diffuse glioma
Associated GBM subtype	MES	PN
Frequency	~90%	~10%
Median age	62	44
Median survival	9-15 months	24-31 months
Location	Supratentorial	Predominantly frontal
Necrosis	extensive	Limited

(*Adapted from Louis *et al.* 2016)

Many studies have attempted to group GBM into subtypes based on expression profiling [7-13]. Phillips *et al.* in 2006 suggested three subclasses based on the dominant gene expression patterns of 107 samples using DNA microarray [7]. Afterward, Verhaak et al. described four subtypes of GBM based on a 840 genes profile: classical, proneural (PN), mesenchymal (MES) and neural [8]. Even though there is no exact GBM subgroup definition, there is a clear demarcation between PN and MES subgroup. The MES subtype has been associated with more invasive tumor, which is also displaying more necrosis and angiogenesis. Primary GBM is predominant in the MES group, which is associated with poorer prognosis. In contrast, the PN subgroup is strongly associated with secondary GBM, which has a better prognosis [7]. Interestingly, Pillips *et al.* demonstrated a shift in GBM subgrouping from PN to MES signature upon tumor recurrence [7].

Pediatric brain tumors represent the most common solid tumor in children. Unlike adult brain tumors, where high-grade glioma (HGG) is prevalent, low-grade

tumors predominate in childrens [14]. Pediatric HGG (pHGG), which comprises WHO grade III astrocytoma and grade IV GBM, represents approximately 20% of all pediatric CNS tumors [15, 16]. Further clear distinctions between brain tumors in adult and children reside in their natural history, location and mutation burden. Most pHGG develops *de novo* and secondary GBM is rare [17]. pHGH commonly arises from cortex hemispheres as in adults. However, cerebellar midline structures such as cerebellum, brainstem, thalamus and spinal cord also represent common sites for brain tumors in childrens but are rarely found in adult brain tumors [18-22]. Diffuse intrinsic pontine glioma (DIPG) represents about half of all pHGG, which is located in the ventral pons in the brainstem. DIPG is associated with the worse prognosis of all pHGG, with less than 10% survival at 2 years, compared to 30% for other pHGG. Anatomical location of DIPG renders adequate drug delivery challenging and surgical resection impossible [23]. Wu *et al.* have identified a recurrent mutation in histone H3 (K27M) in about 80% of DIPG, which provided a clear distinction from pediatric cortical HGG [24]. This mutation was also found in pHGG in other midline structures. In the 2016 WHO CNS classification, DIPG was included in a novel entity termed diffuse midline glioma H3K27M-mutant. This newly defined group of tumors primarily found in the pediatric population is characterized by a diffuse growth pattern, a midline location and a K27M mutation on histone H3 [4, 24, 25]. For the purpose of this review, all pediatric glioma will be referred as pHGG.

2. The Cell of Origin

In most cancers, the quest for the identity of the tumor-initiating cell (TIC) is the Holy Grail as it may provide new avenues to develop efficient therapies. As opposed to the cancer stem cell (CSC) that ensures the growth of the tumor mass, the TIC or cell of origin of tumor designates the normal tissue-resident cell that acquires the initial transforming genetic hit(s). In theory, for glioma, this cell should be one laying at neurogenic regions (i.e. neural stem cell (NSC), or progenitor) or a dedifferentiated cell. While NSCs are quiescent cells displaying extensive self-renewal and multipotential potential, neural progenitors are more committed cells with limited proliferation potential. In the mammalian brain, the subventricular zone (SVZ) carpeting the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus represent the main neurogenic regions [26, 27]. In these locations, both stem and progenitor cells were characterized based on phenotypical markers [28-30]. Oligodendrocyte progenitor cells (OPC) are another pool of unipotent progenitors broadly dispersed in the adult central nervous system. They account for 5% of all brain cells and give rise to mature oligodendrocytes throughout life in both rodents and humans [31-33]. The intrinsic regenerative capabilities of these cell populations and the histological locations of glioma make these cells legitimate candidates as the cell of origin. Using an elegant genetic strategy and a panel of selective markers, these cells were categorized as NSCs ($GFAP^+/NES^+/CNP^-/OLIG2^-/SOX2^+$), early glial progenitor cells (GPCs) ($GFAP^+/NES^+/CNP^-/OLIG2^+/SOX2^+$) or OPCs ($GFAP^-/NES^-/CNP^+/OLIG2^+/SOX2^+$) [34].

Both *in vitro* and *in vivo* experimental models have been developed to mimic and conceptualize initial events of glioma cell transformation. On one hand, NSC and astrocyte cultures were established from Ink4a/Arf- or Tp53-deficient mice. Transduction with constitutively active EGFRvIII, myr-AKT or RAS, overexpression of MYC or activation of PDGFRα,

conferred malignant traits to these cells, with characteristics reminiscent of HGG. In addition, transplanting these cells into immunosuppressed mice led to glioma-like lesions growth [35-38]. On the other hand, genetically engineered mouse models have been proven a powerful tool in searching for the cell at the origin of glioma development. These models involve loss-of-function in tumor suppressor genes or gain-of-function in oncogenes. Pioneer evidence involving SVZ-resident NSCs as the cells of origin in glioma came from the RCAS/tv-a system-based studies [39]. This system allows targeted induction of constitutively active forms of oncogenes under the control of tv-a in astrocytes (GFAP-tv-a; Gtv-a) or in neuroglial progenitors (Nestin-tv-a; Ntv-a). In this experimental setup, only the Ntv-a line developed glioma-like lesions, suggesting that cells within the SVZ may represent the cell of origin. Using the same experimental setup in *Ink4a/Arf*-deficient mice resulted in the formation of glioma-like tumors in GFAP+ cells (Gtv-a/Ink4a-Arf-/- line) [39]. More recently, by using an identical system targeting 3 distinct cell populations (NSCs and OPCs at the SVZ, and GPCs at the retrosplenial cortex), transgenic mice developed glioma lesion displaying different malignancy grades [34]. Glioma-like tumors also developed from progenitor cells located in the brain white matter after *in situ* exposure to PDGF-expressing retroviruses [40]. Stereotactic-guided injection of adenoviruses carrying Cre recombinase into the SVZ of adult mutant mice containing conditional tumour suppressor alleles of *Nf1*, *Trp53*, and *Pten* has been shown to induce glioma-like lesions [41-44]. Moreover, animal models using inducible creER strategies (*Nestin*-, *GFAP*-, *Ascl1*- or *NG2*-directed), exclusively or concomitantly targeting NSCs, GPCs or OPCs gave rise to high-grade glioma-like lesions in close association with the proliferative neurogenic niches and discrete cortical zones [41, 45-47]. At last, concurrent *p53/Nf1* mutations were initiated sporadically by usage of Mosaic Analysis with Double Markers (MADM) system in mice [38]. In this model, sibling mutant and wild-type cells are labeled with different fluorescent markers (GFP vs. RFP). MADM-based lineage tracing revealed aberrant growth followed by malignant transformation of OPCs, but not of NSCs or other neural progenitors, suggesting OPCs as the cell of origin in this model even when initial mutations occur in NSCs.

Although still controversial and based on the aforementioned mouse models, it is worth to note that brain post-mitotic cells (i.e. neurons and astrocytes) could represent TICs as these cells share several markers with brain stem and progenitor cells (i.e. Nestin, GFAP). In this regard, Nestin-expressing neural cells were observed in rodent and human adult brain at four distinct sites. Class I cells are among the smallest neural cells in the brain and are widely distributed. Class II cells are located in the walls of the aqueduct and third ventricle. Class III cells, which co-express markers associated exclusively with neurons, are observed only in the hippocampus and corpus striatum. Class IV cells are found throughout the forebrain and typically reside immediately adjacent to a neuron [48]. Astrocytes are another potential TICs as they share GFAP expression with NSCs [46]. Aside from the SVZ, large astrocytes were detected in the hippocampus and the striatum [49]. This suggests that most cerebral differentiated cells experiencing defined genetic alterations might undergo dedifferentiation to generate a state leading to tumor initiation [50].

Nowadays, the cell at the origin of gliomas remains a topic of controversy. Diverse cell populations in the adult brain have proven to be the primer to glioma.

Unequivocally determining the identity of these cells may open new gates for developing targeted therapies for this devastating disease.

3. Glioma Stem Cells

Growing experimental evidence supports the presence of a small pool of cells, in the tumor bulk, displaying stem cell-like features. These cells are responsible for tumor growth and maintenance [51, 52]. First described in hematopoietic cancers [53-55], CSCs were described within solid tumors as well. Cumulating efforts have proven the presence of such cells in different solid tumors (i.e. breast, brain, colon, skin) [56-60]. These cells are involved in tumor recurrence, metastatic process, and resistance to treatments [61-63]. CSCs were characterized based on cellular properties, and phenotypical markers expression [64].

In glioma, CSCs were first reported following cell cultures from human biopsies. Using stringent culture conditions, a single clone with NSC properties developed as a floating sphere that could be maintained through serial passages [65-67]. *In vivo*, these cells have the capacity to regenerate orthotopic glioma in immunodeficient mice showing similar characteristics as the primary tumor [68, 69]. Under conditions promoting differentiation, these cells display multipotency as they give rise to both neurons and glia, and sometimes differentiate into abnormal cells with multiple differentiation markers in a way that reflects the tumor of origin [65, 68]. These cells express transcripts characteristic of neural and other stem cells (i.e. *CD133/PROMININ, SSEA1/CD15, Nestin, SOX2, GLI2, MUSASHI1, BMI1, LHX2, CD90/Thy1, α6-integrin*) (**Figure 1**) [64, 65, 70-73]. It should be stressed that the use of a combination of markers is necessary to identify glioma CSCs as; (i) single markers are also expressed by NSCs, progenitors and

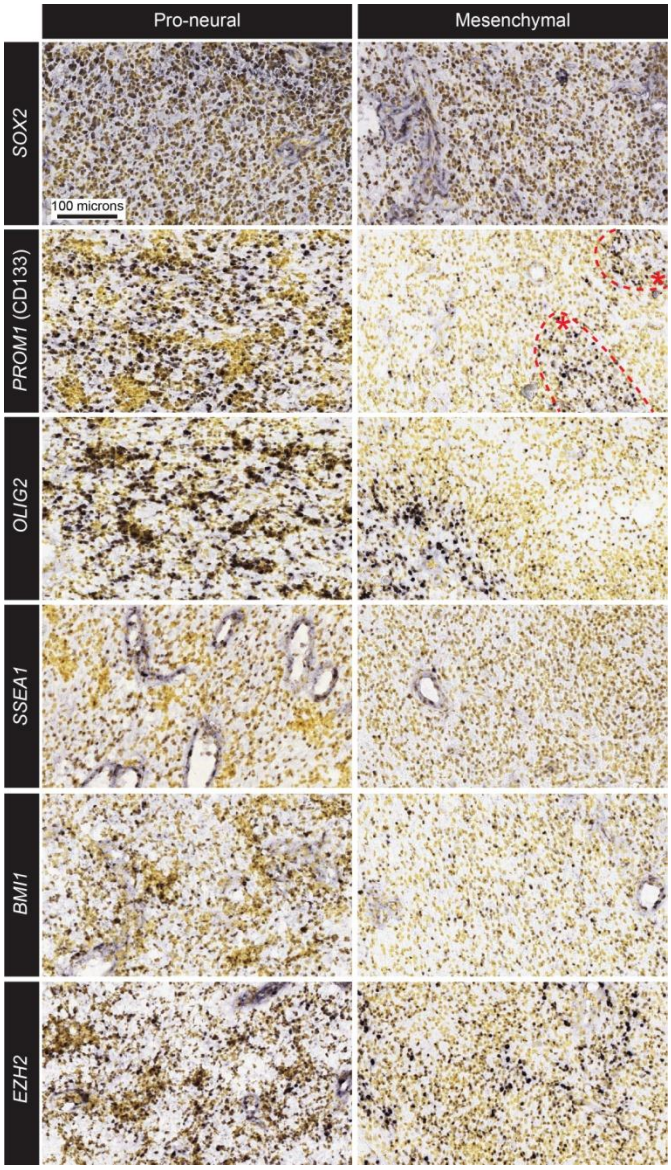

Figure 1. Stem Cell Markers in Glioma
Comparative gene expression analysis using RNA *in situ* hybridization on human glioma is showed (brown signal). One tumor displays a pro-neural phenotype and the other a mesenchymal phenotype. Note the co-expression of most markers in both tumors, but with preferential enrichment of *PROM1*, *BMI1* and *EZH2* in the pro-neural sample. The dashed red lines indicate *PROM1* expression heterogeneity within the mesenchymal tumor, suggesting the presence of multiple tumor clones. Data were extracted from the Ivy Glioblastoma Atlas Project (Allen Institute).

even post-mitotic neural cells, (ii) expression of some of these markers may be lost owing to epigenetic silencing, and/or (iii) their expression may vary between glioma subtypes. In this regard, the unequivocal identification of glioma CSCs is a must as they form the entity that continually fuels the tumor mass, and they are responsible for resistance to radiation therapy and chemotherapy [74, 75]. In addition, these cells could hold for a valuable screening platform for new therapeutics. In fact, and in contrary to glioma cell lines, histological and gene expression pattern analyses have shown a strong correlation between the original patient tumor and tumors derived from CSCs [66].

4. Genetics

Inhibition of tumor suppressors and activation of cell proliferation through oncogenes represent the main limitation events in cancer development. Induction of angiogenesis, DNA dammage repair, invasion and drug resistance mechanisms are often responsible for the proliferation of high grade tumors that are extremely challenging to treat and more prone to recurrence.

In the adult population, TP53, RB1, NF1, CDKN2A and PTEN are all tumor suppressors found to be deleted or mutated in glioma. Inactivation of the p53 pathway was also delineated by ARF deletions and MDM2 or MDM4 amplifications [76]. Aberrations in the RB pathway was mostly caused by deletion of CDKN2A/CDKN2B followed by CDK4 locus amplification. Dysregulation of growth factor signaling through amplification and activating mutations of receptor tyrosine kinases (RTKs) also represent an important genetic event. Aberrant expression of proto-oncogenes such EGFR, ERBB, PDGFRA, MET and BRAF has been commonly identified (**Figure 2**) [77]. Activation of MGMT through mutation and/or methylation of CpG islands within the promoter enable a drug resistance mechanism. MGMT removes the methylations introduced by temozolomide, a common chemotherapy drug, thereby allowing DNA damage repair [78, 79]. Isocitrate dehydrogenase 1 and 2 (IDH) mutations have been observed in the majority of low-grade gliomas and secondary GBM. These mutations have influenced the recent 2016 WHO classification for CNS tumor [80-84]. IDH1 mutation results in accumulation of the metabolite 2-hydroxyglutarate (2-HG), which impairs the activity of TET, a methylcytosine dioxygenase, is reflected in DNA hypermethylation. Mutations in IDH1 indirectly influence H3K27 or H3K36 methylation via 2-HG, which can impair cell differentiation [85-87].

In contrast, pHGG is linked to a limited number of driver mutations that account for aberrant DNA copy number and altered gene expression (**Figure 2**) [88-94]. These tumors display aneuploidy, gene rearrangements and amplifications, and chromosomal gain or loss (i.e. 1q gain, 7 gain and 10q loss in 30%, 13% and 35% of tumors, respectively). Detailed mapping revealed numerous focal amplifications of genes within RTK signaling pathway and cell-cycle regulatory genes (i.e. IGF1R, PDGFRB, PDGFRA, MET, PIK3CA, TP53, CDK6, CCND1, CCNE1, PARP1), recurrent activating somatic mutations of the receptor serine/threonine kinase ACVR1/ALK2, recurrent gene fusions involving the neurotrophin receptor genes NTRK1, NTRK2 and NTRK3, and homozygous deletions affecting still to date unknown genes (i.e. homing at 5q35, 10q25, and 22q13). A unique feature however of pHGG is the presence of somatic mutations affecting histone H3 [24, 91].

Specific recurrent mutations in *H3F3A* (encoding histone H3.3) or in the related *H3B/3C* (encoding histone H3.1) led to amino acid substitutions at two critical positions (namely positions 27 and 34) within the histone tail (K27M and G34R/V) involved in key regulatory post-translational modifications. These mutations were found to be specific to pHGG and highly prevalent in children and young adults. In addition, mutations were found in ATRx and DAXX [24, 91].

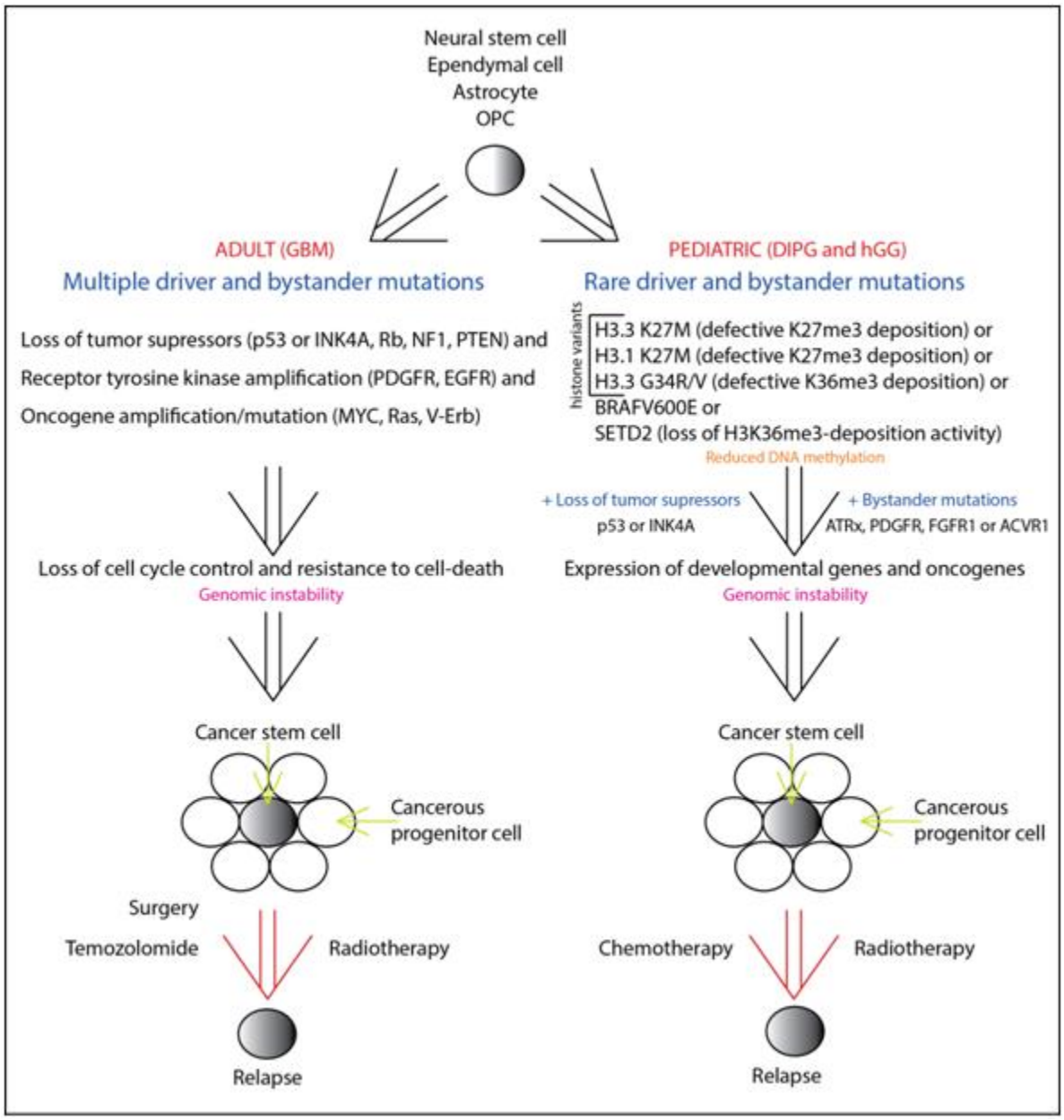

Figure 2. The road to Cancer in Glioma

Schematic representation of the sequential genetic and epigenetic modifications leading to gliomagenesis is showed. In this model, acquisition of the cancer stem cell phenotype is responsible for tumor relapse after radiotherapy and chemotherapy treatments.

Interestingly, histone mutations segregate frankly depending on the anatomical distribution of tumors. H3.3G34R/V mutation is found exclusively in the cerebral hemispheres, H3.3K27M mutation is found throughout the midline structures (including the thalamus, brainstem, cerebellum, and spine) and H3.1K27M mutation is restricted to the pons. In addition, other mutations might categorize these subtypes (ACVR1 exclusive to H3.1K27M mutation, and CDKN2A/B, EGFR and FGFR1 exclusive to H3.3-associated mutations) [18, 93]. A small and distinct biological subgroup of pHGG also harbors hotspot mutations affecting IDH1, BRAF (V600E), MYCN or MYC (**Figure 2**) [93, 95, 96]. Ongoing studies are on to classify a subset of tumors that present free of the aforementioned aberrations. Nonetheless, regarding these childhood diseases, there are clear differences in location, histology and driving epigenetic and genetic alterations. It emerges that stratification of pHGG will likely be valuable to develop new and efficient targeted therapies.

5. Epigenetics

5.1 The Chromatin

Chromosomes are structurally organized in distinct sub-compartments as determined by the local DNA sequence and chromatin organization. Euchromatin defines "relaxed" chromatin regions containing actively transcribed genes. In contrast, heterochromatin defines "compacted" chromatin regions containing tissue-specific and developmental genes (the facultative heterochromatin) or gene-poor repetitive DNA sequences found at centromeric, pericentromeric and telomeric parts of chromosomes (the constitutive heterochromatin) [97, 98]. Nucleosomes are the basic building unit of chromatin and are constituted of a 147 bp strand of DNA wrapped against a histone octamer containing two molecules of each of the four histones H2A, H2B, H3 and H4 (the nucleosome core particle) [98]. The addition of linker histones, such as histone H1, increases the amount of associated DNA by 20 bp to elicit higher levels of chromatin compaction and high order chromatin structure. Post-translational modifications of histones tail, such as methylation, acetylation and ubiquitylation can modify chromatin compaction and stability. Silent chromatin is generally but not exclusively associated with tri-methylation of histone H3 at lysines 9 ($H3K9^{me3}$) or 27 ($H3K27^{me3}$), while transcriptionally active chromatin is associated with histone H3 tri-methylation at lysine 4 ($H3K4^{me3}$) or acetylation at lysines 9 ($H3K9^{ac}$) or 27 ($H3K27^{ac}$) [97].

5.2 Histone H3

The finding that a large proportion of pHGG carry mutations in histone H3 brought renewed interest for the study of histone modifications in brain cancer [91]. There are 7 variants for histone H3. The canonical histones H3.1 and H3.2 are encoded by multiple identical genes, allowing a high level of transcription. Histones H3.1 and H3.2 can be replaced by non-canonical histones, which differ by 1-5 amino-acids at critical residues [99]. In contrast to canonical histones which are incorporated *de novo* during DNA replication, histone H3.3 is incorporated in all cell cycle phases, including G0 [99]. This unique feature is thought to be of major importance in post-mitotic neurons and in the context of aging and senescence [99]. In dividing cells, histone H3.3 is preferentially located in "open" chromatin regions and is also abundant in repeat-rich chromatin regions such as pericentric heterochromatin and in telomeres where it is trimethylated at lysine 9 (H3.3K9me3) and loaded by the

ATRx/DAXX chaperone complex [100, 101]. Histone H3.3K9me3 also inhibits endogenous retrovirus activation in mouse embryonic stem cells [102]. The ATP-dependent chromatin remodeler ATRx localizes at both telomeres and peri-centromeric heterochromatin, and germline mutations in *ATRx* are associated with the Alpha-thalassemia with mental retardation X-linked syndrome [103-105].

Loss of function mutations in *ATRx* have been identified as "bystander mutations" in pediatric gliomas, leading to genomic instability, and are also involved in the Alternative Lengthening of Telomere (ALT) phenotype of telomerase-independent cancers [91, 105, 106]. ATRx co-precipitates with the Polycomb group protein B cell-specific Moloney murine leukemia virus Integration site 1 (BMI1) and histone H3K9me3, and both ATRx and BMI1 are enriched at constitutive heterochromatin regions [107].

5.3 Polycomb Group Proteins

Polycomb group proteins form large multimeric complexes involved in gene silencing through modifications of chromatin organization [108]. They are classically subdivided into two groups, namely Polycomb Repressive Complex 1 (PRC1) and PRC2 [109]. The sequential histone modifications induced by the PRC2 complex (which includes EZH2, EED and SUV12) and the PRC1 complex (which includes BMI1, RING1a, and RING1b/ RNF2) allows stable silencing of gene expression in euchromatin and facultative heterochromatin [110-112].

The PRC2 contains histone H3 tri-methylase activity at lysine 27 (H3K27me3) and the PRC1 contains histone H2A mono-ubiquitin ligase activity at lysine 119 (H2Aub) [110-112]. During mouse development, H3K27me3 deposition by PRC2 is thought to occur before and be required for PRC1 recruitment at developmental genes. In somatic cells however, the H2Aub mark may be required for PRC2 complex maintenance and H3K27me3 deposition on the chromatin, thus creating a positive feedback loop [113]. A number of observations have implicated these proto-oncogenes in human cancers [114-119]. BMI1 is part of the PRC1 and was originally identified as an oncogenic partner in lymphomagenesis. BMI1 was later found to be overexpressed in several cancers and important for cancer cell survival in medulloblastoma and glioblastoma [70, 120-126]. BMI1 knockdown in human GBM cells resulted in loss of CSC self-renewal and absence of tumor formation in grafted mice [70]. Interestingly, BMI1 was also reported to be over-expressed in cultured neurospheres from childhood brain tumors, including one midline anaplasic astrocytomas (grade III), one medullo-blastoma and one glioblastoma [65].

Hence, BMI1 is over-expressed in 53% of pHGG *in situ* and BMI1 inactivation in cancerous neurospheres impairs tumor formation in mouse xenografts [127]. EZH2 is also expressed in adult GBM and its inactivation impairs cell growth, prompting interest as a potential target against gliomas [70, 128-130].

However, while EZH2 generally works as a proto-oncogene, it can also suppress tumor transformation. Recently, it was showed that acute Ezh2 inactivation in mouse GBM impaired tumor growth and extended lifespan. Surprisingly, prolonged Ezh2 inactivation resulted in loss of the H3K27me3 mark, activation of some pluripotency markers, switch in cell fate and aggressive tumor evolution [131]. Thus, loss of H3K27me3-mediated gene repression is a prognostic for tumor transformation toward a more immature and aggressive cell fate.

5.4 Chromatin Signature in HGG

Since the identification of the H3K27M mutation, Polycomb group proteins became strong candidates as part of the missing link between adult and pediatric glioma. Pioneer work revealed that K27M mutant heterotypic nucleosomes did not sequester PRC2 but rather prevented its binding [132]. Genome-wide, K27M glioma cells displayed highly reduced H3K27me3 levels, which correlated with increased expression of PRC2-repressed target genes (i.e. developmental genes) and of some cancer-promoting loci. On the other hand, PRC2 accumulated at specific H3.3 poor loci, resulting in increased H3K27me3 levels and gene repression[132].

Since H3K27me3 deposition can be reversed by de-methylase activities of the KDM6-family proteins JMJD3 and UTX, it was tested whether inhibition of KDM6 using GSK-J4 could increase H3K27me3 levels and thus impact of tumor growth. Notably, K27M-carrying pHGG, but not wild type pHGG, were found to be specifically sensitive to KDM6 inhibition, thus providing the fist hint for possible application of epigenetic drug therapy [133]. Similarly, Grasso *et al.* screened chemo-therapeutic and epigenetic compounds for their activity on pHGG. They found that the histone deacetylase inhibitor (HDACi) panobinostat was effective at reducing cancer cell growth in part through induction of both H3K27 tri-methylation and H3 acetylation. The combination with GSK-J4 also provided "synergistic" activity against tumor growth *in vitro* and in xenograft mouse models [134].

In K27M-carrying glioma, PRC2 was previously found to accumulate at H3.3 poor loci, resulting in gene repression [132]. One of the loci affected by this phenomena is the *INK4A* locus, the main target of PRC1 and PRC2 repressive activities [135, 136]. The *INK4A* locus is frequently deleted in cancers and encodes for the p16INK4a and p14ARF tumor suppressors, which act on the Rb and p53 pathways, respectively [137]. Notably, EZH2 inhibition with the small compounds GSK343 or EPZ6438 in *INK4A*-proficient pHGG carrying the K27M mutation resulted in significant tumor regression [135]. At the molecular level, it was discovered that H3K27M heterotypic nucleosomes where hyper-acetylated at lysine 27 (H3K27ac), resulting in recru-itment of the BET bromodomain proteins BRD2 and BRD4, of RNA PolII and elevated transcription, reminiscent of the structure found at "super-enhancers". Pharmaceutical inhibition of BET bromo-domain proteins with JQ1 in pHGG resulted in neural differentiation in part through transcriptional down-regulation of "super activated" loci, including *GLI2*, which is known to regulates the activity of the cancer-promoting sonic hedgehog (Shh) pathway [138-141]. Likewise, pHGG carrying the G34R/V mutation or *SETD2* loss-of-function have reduced levels of H3K36me3, which correlated with activation of developmental genes and of *MYCN* [142, 143], altogether suggesting the presence of a common "chromatin signature" for pHGG.

5.5 The Chromatin Hub

Although adult and pediatric glioma have variable developmental and cellular origins and carry distinct combinations of genetic anomalies, yet they may share a common "chromatin hub", as suggested by their mutual relationship with Polycomb group proteins (**Figure 3**). Many transcription factors (TFs), such as SOX2 and OLIG2, are found in glioma and operate as core TFs for gliomagenesis and tumor maintenance [43, 65, 144]. By analogy to the reprogramming of somatic cells into pluripotent stem cells with a core of 4 master TFs [145], it was shown that over-

expression of SOX2, OLIG2, POU3F2 and SALL2 in differentiated GBM cells could re-instate the CSC molecular program and drive tumor formation [146]. Likewise, ectopic expression of SOX2, OLIG2 and ZEB1 was found sufficient to convert tumor suppressor-deficient astrocytes into glioma-initiating cells, and this independently of RTK amplification or gain-of-function mutations [147]. Interestingly, previous work showed that Bmi1 over-expression was sufficient to "reprogram" mouse astrocytes into neural stem cells [148] or mouse retinal progenitors into retinal "stem cells" by conferring self-renewal properties [149].

Additional TFs and chromatin remodelers, such as TLX, ZFHX4 and MLL5, were found to be abundant in glioma CSCs and required for their self-renewal [150-152]. While the intricate relationship between these factors in CSC biology remains to be elucidated, one can speculate that they form a network of self-reinforcing TFs that confer CSC identity in part by repressing alternative cell fates.

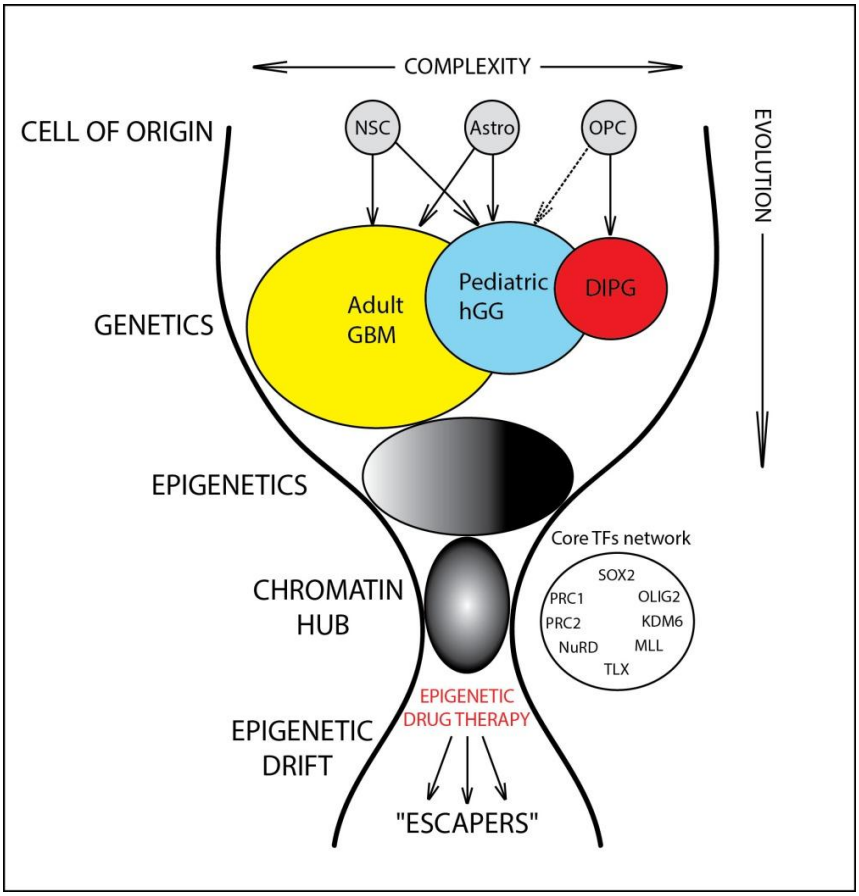

Figure 3. The Chromatin Hub

Model of the glioma genetic and epigenetic complexity and evolution over time is showed. While genetic complexity within groups and between groups is very large, epigenetic complexity tends to diminish, to ultimately reach the least variable constriction point referred to as the Chromatin Hub. The Chromatin Hub is defined by the presence of nework of core transcription factors (TFs) that are essential to maintain the self-renewal properties of cancer stem cells. Targeting Chromatin Hub's components holds therapeutic promises against glioma but at a risk of inducing an irreversible epigenetic drift of the tumor.

This led to the proposition that epigenetic circuits superimposed upon genetic mutations determine key features of cancer cells, and possibly the CSC phenotype [146]. Notably, glioma cells treated with RTK inhibitors can rapidly adapt by adopting a "persister-like" state involving *EZH2* downregulation and *KDM6* upregulation, re-distribution of repressive chromatin marks and activation of developmental genes [153]. This situation is reminiscent of the chromatin phenotype of pHGG carrying the K27M or G34R/V mutations. Interestingly, *MLL5*, the most divergent member of the mixed lineage leukemia (MLL) gene family, is highly expressed in adult GBM. MLL5 represses *H3F3B* transcription (encoding for H3.3) and this preferentially in CD133+ cells. Inactivation of MLL5 increased H3.3 levels, leading to loss of specific (yet uncharacterized) heterochromatin domains and deficiency in CSC self-renewal through differentiation [152]. Thus, MLL5 antagonizes H3.3 accumulation (and by inference H3.3K27me3 accumulation) to promote CSC self-renewal, which is also reminiscent of pHGG with H3.3K27M mutation.

The implication of these findings is that despite their notable divergences in mutational spectrum, developmental time-point and cellular origin, adult and pediatric glioma may both depend on a common "chromatin hub" that is critical to sustain CSC self-renewal (**Figure 3**). Targeting chromatin hub components may be the key to cure disease.

6. Therapeutic Perspectives

Despite numerous efforts, the treatment of glioma remains highly challenging and has poorly evolved in the past decades. Epigenetic therapies for pHGG using KDM6, HDAC, BET or EZH2 inhibitors show promises in mouse models, but still have to show efficacy in patients (reviewed in [154, 155]). Combined epigenetic therapy using panobinostat and GSK-J4 apparently displays synergistic effect at suppressing tumor growth [134]. GSK-J4 was also reported to inhibit the colony formation capacity of freshly isolated adult GBM cells *in vitro* [152]. However, none of these treatments were shown to cure the disease in mouse models, raising concern about their possible translational application to humans.

BMI1 is implicated in DNA damage response and maintenance of genomic stability, which may open new avenues for multimodal therapies [121, 156-158]. Recently, this dual strategy proved to be effective in a mouse model of head and neck squamous cell carcinoma where BMI1 inhibition sensitized otherwise resistant CSCs to cisplatin treatment and eliminate lymph node metastasis and tumor bulk [159].

Notably, a BMI1 inhibitor has been tested in human colorectal tumors containing CSCs and was shown to inhibit cell growth and improve lifespan in grafted animals [160]. Notably, panobinostat may sensitize cells to DNA damage since it was shown to display significant therapeutic activity only when combined with radiation treatments in clinical assays [154]. Thus, combinatorial therapies involving epigenetic drugs and classical DNA-damaging agents may synergize to eliminate CSC and cure the disease. Also, since epigenetic therapy can promote epigenetic drift (**Figure 3**) [131], combination with another epigenetic agent blocking the alternate differentiation program together with radio- or chemo-therapy may prove extremely effective. Extensive characterization of the cancer cell response to these epigenetic compounds is thus a pre-requisite to an intelligent design of drug combinations and treatments.

Acknowledgments

This work was supported by a grant from the Cancer Research Society (G.B.), and fellowships from the Programme de Biologie Moléculaire de l'Université de Montréal (A.F.) and Hôpital Maisonneuve-Rosemont (R.H.).

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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