UCSF

UC San Francisco Previously Published Works

Title

The role of histone modifications and telomere alterations in the pathogenesis of diffuse gliomas in adults and children

Permalink

https://escholarship.org/uc/item/6z64q1kc

Journal

Journal of Neuro-Oncology, 132(1)

ISSN

0167-594X

Authors

Lee, Julieann
Solomon, David A
Tihan, Tarik

Publication Date

2017-03-01

DOI

10.1007/s11060-016-2349-9

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

HHS Public Access

Author manuscript

J Neurooncol. Author manuscript; available in PMC 2018 March 01.

Published in final edited form as:

J Neurooncol. 2017 March ; 132(1): 1–11. doi:10.1007/s11060-016-2349-9.

The Role of Histone Modifications and Telomere Alterations in the Pathogenesis of Diffuse Gliomas in Adults and Children

Julieann Lee, **David A. Solomon**, and **Tarik Tihan**

Division of Neuropathology, Department of Pathology, University of California, San Francisco

Abstract

Genetic profiling is an increasingly useful tool for sub-classification of gliomas in adults and children. Specific gene mutations, structural rearrangements, DNA methylation patterns, and gene expression profiles are now recognized to define molecular subgroups of gliomas that arise in distinct anatomic locations and patient age groups, and also provide a better prediction of clinical outcomes for glioma patients compared to histologic assessment alone. Understanding the role of these distinctive genetic alterations in gliomagenesis is also important for the development of potential targeted therapeutic interventions. Mutations including K27M and G34R/V that affect critical amino acids within the N-terminal tail of the histone H3 variants, H3.3 and H3.1 (encoded by *H3F3A* and *HIST1H3B* genes), are prime examples of mutations in diffuse gliomas with characteristic clinical associations that can help diagnostic classification and guide effective patient management. These histone H3 mutations frequently co-occur with inactivating mutations in *ATRX* in association with alternative lengthening of telomeres. Telomere length can also be maintained through upregulation of telomerase reverse transcriptase (TERT) expression driven by mutation within the *TERT* gene promoter region, an alteration most commonly found in oligodendrogliomas and primary glioblastomas arising in adults. Interestingly, the genetic alterations perturbing histone and telomere function in pediatric gliomas tend to be different from those present in adult tumors. We present a review of these mutations affecting the histone code and telomere length, highlighting their importance in prognosis and as targets for novel therapeutics in the treatment of diffuse gliomas.

Keywords

histone H3.3; *H3F3A*; *HIST1H3B*; *ATRX*; *DAXX*; *TERT*; telomerase; alternative lengthening of telomeres (ALT); diffuse glioma; oligodendroglioma

Packaging Agents and Keepers of DNA Order: HISTONES

Histones are highly alkaline nuclear proteins that perform critical functions in packaging and organizing DNA into structural units to maintain gene regulation. Histone octamers are the protein core of nucleosomes, and contain two each of the H2A, H2B, H3, and H4 subunits (Figure 1). These subunits also contain N-terminal “tails” which constitute the location of

To whom correspondence should be addressed: Dr. Tarik Tihan, MD, PhD, Division of Neuropathology, University of California, San Francisco, 505 Parnassus Ave, Room M-551, Box 0102, San Francisco, CA 94143, Phone: 415-476-5236, Fax: 415-476-7963, tarik.tihan@ucsf.edu.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

most post-translational modifications, alternatively known as marks, and include acetylation, mono-, di-, or tri-methylation, phosphorylation, and ubiquitination. According to the "histone code" hypothesis, the location and combination of tail marks has significance for eliciting downstream effects on cellular functions such as DNA replication, chromosome condensation and mitosis, DNA repair, maintenance of centromeres and telomeres, and gene expression [1, 2]. This histone code is maintained by code writers, erasers, and readers; these are enzymes that place, remove, or interpret histone tail modifications.

In addition to post-translation modifications, nucleosomes can alter gene expression through incorporation of conserved histones variants called replacement histones. While the transcription of canonical histone proteins is tightly coupled to DNA replication during S phase, replacement histones are expressed throughout the cell cycle and are deposited into nucleosomes in a replication-independent manner.

Histone 3.1 is the canonical histone 3 (H3) protein, encoded by ten genes of the *HIST1* cluster on chromosome 6, including the *HIST1H3B* gene [3]. Histone 3.3 (H3.3) is a replacement histone, encoded by *H3F3A* or *H3F3B*, unique genes on chromosome 1 and 17 producing an identical amino acid sequence [4, 5]. H3.3 varies from H3.1 by five amino acids (codons 31, 87, 89, 90, and 96) with amino acid 31 being a serine of the H3.3 N-terminal tail that can be phosphorylated [5]. The incorporation of H3.3 into telomeric and pericentric chromatin is accomplished by ATRX-DAXX (alpha thalassemia/mental retardation syndrome X-linked and death-domain associated protein) chaperone protein complex, while HIRA (histone regulator A) deposits H3.3 into chromatin areas of active and suppressed genes [6, 7, 8, 9].

Protectors of Chromosomes and Cellular Longevity: TELOMERES

Telomeres provide the solution to the universal "end replication problem," which occurs in every cell due to inability to completely replicate chromosome ends [10, 11, 12, 13]. Telomere length can be maintained by the enzyme telomerase that contains a telomerase reverse transcriptase (TERT) catalytic component and an RNA template strand. While telomerase expression and activity is maintained within stem cells by epigenetic mechanisms, telomerase activity is minimal to undetectable in most somatic cells [11, 13, 14]. Two hotspot mutations in the promoter region of the *TERT* gene (chr.5: g. 1,295,228C>T and g.1,295,250C>T) were first reported in 71% of sporadic melanomas [15, 16], and have since been detected in numerous cancers [14]. These mutations create novel-binding sites for E-twenty-six (ETS) transcription factors, specifically the GABP transcription factor that binds to the mutant promoter, resulting in increased TERT transcription [17].

In the absence of telomerase activity, telomere length can be maintained by homologous recombination, a mechanism known as alternative lengthening of telomeres (ALT). ALT is utilized by approximately 5–15% of human cancers [18, 19], and is associated with genetic inactivation or loss of expression of the histone H3.3 chaperone proteins ATRX and DAXX [19, 20].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Mutations of histone genes and readers, writers, and erasers of the histone code in gliomas

Mutations in genes affecting the histone code and alterations of histone methylation status have been reported in several cancer types [2, 21, 22, 23, 24, 25, 26, 27]. However mutations directly affecting histone genes were originally described in gliomas [1, 28, 29, 30], and have been subsequently found in chondroblastomas and giant cell tumors of bone [6, 31]. Tail mutations of histone H3 at amino acids 27 and 34 leading to K27M (lysine to methionine substitution at codon 27) and G34R/V (glycine to arginine or valine substitution at codon 34) were initially reported in 36% of non-brainstem pediatric high-grade gliomas, with 14% containing G34R/V mutations and 22% containing K27M mutations [29] (Table 1). H3.3 K27M mutation is also found in 70–80% of pediatric diffuse intrinsic pontine gliomas (DIPGs) [28, 29] (Figure 2), a tumor now classified as diffuse midline glioma, H3 K27M mutant, WHO grade IV. Mutations predominantly occur in the *H3F3A* gene encoding H3.3, but analogous K27M mutations are also found at lower frequency in histone 3.1 gene *HIST1H3B*. Histone H3.3 K27M and G34R/V mutations are mutually exclusive with each other, with analogous histone 3.1 mutations, and with *IDH1/2* mutations [29, 30, 32]. H3 K27M mutations characterize diffuse midline gliomas arising in both pediatric and adult patients, while H3 G34R/V mutations are found in diffuse gliomas centered within the cerebral hemispheres arising predominantly in older pediatric patients [30].

Diffuse midline astrocytomas containing somatic H3 K27M substitution mutations were initially reported within the thalamus, pons, and spinal cord of pediatric patients at an average age of 10 years and correlated with worse prognosis compared to patients with histone H3 wild-type tumors [1, 28, 29, 30]. The occurrence of H3 K27M mutations has now been expanded to additional midline locations including the third ventricle, hypothalamus, pineal region, and cerebellum [33]. While pontine K27M mutations are found in younger patients, K27M mutations have been increasingly recognized in diffuse midline gliomas of adolescents and adults, occurring in 52–58% of adult spinal cord, brainstem, and thalamic gliomas [34, 35]. Diffuse gliomas with K27M mutations have been reported in the thalamus and spinal cord with a median age of 24 years, and in the pineal region of a 65-year-old patient [33]. In pediatric diffuse midline gliomas, K27M mutation status correlates with very poor prognosis regardless of histologic grade, whereas K27M mutation in thalamic gliomas of adult patients does not always correlate with worse prognosis [3, 28, 35, 36]. Diffuse midline glioma, H3 K27M mutant, is defined within the 2016 WHO Classification of Tumours of the Central Nervous Systems as a grade IV neoplasm even in the absence of high-grade histologic features.

ATRX or *DAXX* gene mutations have been reported to co-occur with K27M mutations, yet the frequency of co-occurrence is variable across studies with reported co-mutation rates of 30%–60% [1, 37]. Variability in the coexistence of *ATRX* and K27M mutations may be explained by location specific differences. The co-mutation rate was recently reported as 75% in thalamic gliomas, 57% in spinal gliomas, and 8% in pontine gliomas [33].

G34R/V histone H3 mutations are found in diffuse gliomas within the cerebral hemispheres, affect adolescents with a median age of 18, and portend a better prognosis compared to

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

diffuse midline gliomas with H3 K27M mutation [1, 30]. *ATRX* mutations frequently occur with G34R/V histone mutations, with reported co-mutation rates ranging from 75% to 100% [1, 37]. Diffuse gliomas arising in the cerebral hemispheres can alternatively harbor mutations in *SETD2*, a methyltransferase specific to lysine-36 of the histone 3 tail, making the encoded SETD2 (SET domain containing 2) protein a histone code writer. *SETD2* mutations have been reported in approximately 15% of pediatric high-grade diffuse gliomas in the cerebral hemispheres. *SETD2* mutations often occurred in children above the age of 12 and had very frequent co-occurring *ATRX* mutations, with similar age range and co-mutation spectrum as G34R/V mutant gliomas [38]. Mutations in *SETD2* were mutually exclusive with H3 G34R/V mutations in all gliomas studied to date.

Mutations affecting the histone code of diffuse gliomas include the following: (1) mutations of the histone tail that directly alter a post-translational modification site, exemplified by the K27M mutation, (2) mutations within the histone tail that are adjacent to and interfere with a post-translational modification site, namely the G34R/V mutations, (3) mutations within enzymes or that affect enzymes involved in writing or erasing the histone code, such as *SETD2* mutation or *IDH1/2* mutation (Figure 1).

The heterozygous K27M mutation in histone H3 impairs methylation at position 27 by two mechanisms. Methionine does not undergo methylation; this substitution directly removes a methylation site from the histone tail. Additionally, the K27M-mutant histone 3.3 protein is a dominant-negative inhibitor of K27 methylation by sequestration of the PRC2 complex (polycomb repressive complex 2), which contains the EZH2 (enhancer of zeste) K27 methyltransferase. This results in decreased levels of tri-methylated lysine 27 of histone 3 (H3K27me3), a histone tail mark typically associated with silenced chromatin [32, 39].

Diffuse gliomas with G34R/V and K27M mutations have distinct hypomethylated genomes, a finding most prominent in G34R/V mutations with particularly pronounced hypomethylation at chromosome ends [30, 32]. G34R/V and K27M mutations both show mutation-specific gene expression profiles, with distinctive expression patterns similar to those observed during normal brain development [1, 30, 32]. In particular G34R/V mutant gliomas have gene expression profiles resembling early embryonic and early to mid fetal stages of neocortical and striatum development, while K27M gliomas have gene expression profiles that resemble mid to late fetal stages of thalamic and striatum development [30]. Of note, the PRC2 complex that is inhibited by K27M mutation additionally plays a prominent role in the progression of neural precursor cell differentiation. PRC2 within neural precursor cells suppresses neuronal differentiation and promotes astrocytic differentiation [40]. While the exact cell of origin is unknown, K27M and G34R/V mutant gliomas are thought to have a different cell of origin due to their unique gene expression profiles and location predilections [30]. The mechanistic link between altered histone tail methylation, DNA hypomethylation, and altered gene expression is yet to be elucidated.

While G34R/V mutations do not directly occur at a site of post-translation modification, they occur in close proximity to lysine 36 of the histone H3.3 tail, which can be mono-, di-, and tri-methylated or acetylated in association with transcriptionally active or silenced chromatin. As glycine is a small uncharged amino acid residue, substitution with arginine or

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

valine likely alters the post-translational modifications that occur at lysine 36 through steric inhibition or conformational tail changes, thereby interfering with code writers, erasers, and readers [1, 6]. Decreased histone H3 K36 trimethylation (H3K36me3) is observed within nucleosomes that contain an H3.3 G34R/V mutated tail. Furthermore, diffuse gliomas in the cerebral hemispheres lacking histone H3 gene mutations but instead harboring inactivating *SETD2* mutations also demonstrate a significant decrease in H3K36me3 levels [38, 39].

Hotspot mutations affecting the *IDH1* or *IDH2* genes, encoding isocitrate dehydrogenase enzymes of the citric acid cycle, define the vast majority of lower grade diffuse gliomas and secondary glioblastomas arising in adults, but are only rarely seen in gliomas in young children. These *IDH1/2* mutations also affect histone methylation, and in contrast to K27M and G34R/V mutations, are associated with DNA hypermethylation. The recurrent *IDH1* R132H and *IDH2* R172H substitution mutations cause a gain-of-function that results in production of the oncometabolite 2-HG (D-2-hydroxyglutarate), which inhibits a wide range of histone demethylases, including those involved in the demethylation of H3K4, H3K9, H3K27, and H3K79 [41, 42, 43].

Mutations and other alterations in genes affecting telomere length in gliomas

Amongst diffuse gliomas in adult patients, *TERT* promoter mutations are seen in almost all tumors (88–98%) with co-deletion of chromosomes 1p and 19q along with *IDH1* or *IDH2* mutation, the typical molecular profile of adult-type oligodendroglioma [44, 45], which demonstrates better overall survival compared to other glioma molecular groups [46] and elevated TERT expression [47]. Additionally *TERT* promoter mutations have been reported in 58–83% of adult primary glioblastomas, with a lower prevalence in secondary glioblastomas (28%) [14, 44, 48]. In grade II-III adult diffuse gliomas without 1p and 19q co-deletion, only 15% have *TERT* promoter mutations [46]. *TERT* promoter mutations in adult primary glioblastoma are associated with worse prognosis [46, 49]. A high frequency of *TERT* promoter mutations (81%) have been reported in the gliosarcoma variant of glioblastoma [45].

While *TERT* promoter mutations were originally reported in 11% (2/19) of primary pediatric glioblastomas [14], a subsequent study found *TERT* promoter mutations in only 3% (1/32) of pediatric primary glioblastomas [45]. The single case in the second study was a non-canonical g.1,295,228C>A mutation that did not create a novel ETS transcription factor binding site [45]. In general, pediatric gliomas rarely have *TERT* promoter mutations; analysis of 373 pediatric nervous system tumors including 200 gliomas found only 7 *TERT* promoter mutations [45]. Although pediatric *TERT* promoter mutations are rare, a single study has reported *TERT* promoter hypermethylation in association with increased TERT expression in pediatric high-grade gliomas [50]. Aberrant *TERT* promoter methylation with increased TERT expression has been reported in other cancer types [51, 52, 53]. In adult gliomas *TERT* promoter mutation rather than promoter methylation is the predominant mechanism for TERT upregulation [54]. Although *ATRX* and *TERT* promoter mutations are usually mutually exclusive, supporting an equivalent selective advantage of mutations

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

maintaining telomere length through either ALT or increased telomerase activity [14], rare exceptions to this have been observed in adult gliomas [46, 47].

Somatic mutations in *ATRX* and *DAXX* were initially identified in pancreatic neuroendocrine tumors [55] and were found to be highly associated with ALT [20]. *ATRX* or *DAXX* mutations are present in approximately 30% of pediatric glioblastomas, with mutation and loss of immunostaining occurring much more frequently in ATRX than DAXX, and ALT highly correlating with ATRX loss [1]. ALT in high-grade pediatric gliomas with *TP53* mutation is associated with increased overall survival [56]. *ATRX* mutations have also been reported in 9% of DIPGs, but are not prevalent in pediatric low-grade gliomas [28, 57, 58]. ALT rarely occurred in pediatric low-grade gliomas [56], an interesting finding due to the corresponding low prevalence of *ATRX* mutation. This is consistent with the concept that telomere maintenance is not a necessary component of pediatric low-grade gliomas. Lack of telomere maintenance in pediatric low-grade gliomas may explain the occurrence of growth arrest and the low rate of transformation to high-grade gliomas [59].

While *ATRX* mutations in pediatric glioblastomas are associated with *TP53* and histone H3 mutations [1, 56], *ATRX* mutations in diffuse gliomas within adult patients are associated with concurrent *TP53* and *IDH1/2* mutations (Figure 3), occurring in greater than 90% of lower-grade diffuse astrocytomas and secondary glioblastoma in the context of *IDH1/2* mutation with intact chromosomes 1p and 19q [47, 57]. In contrast, *ATRX* mutations are seen in 4–7% of primary glioblastomas with wild-type *IDH1/2* alleles [20, 57, 60]. *DAXX* mutations are rare in adult lower-grade gliomas at less than 1% frequency [61].

ALT is observed in pediatric high-grade astrocytomas (29%), DIPG (18%), adult high-grade (26%), and low-grade (27%) astrocytomas [56, 62]. ALT is highly associated with *ATRX* mutations in adult low-grade gliomas and in high-grade astrocytomas of children and adults [60, 62]. Accordingly there is interest in the role of ATRX in gliomagenesis, and the potential connection with histone mutations. In vitro studies in immortalized cell cultures have shown that ATRX loss by itself is not sufficient to produce ALT, suggesting that additional genetic and/or epigenetic changes are required [18, 19, 63]. This is in agreement with the existence of *ATRX* mutations in adult high-grade gliomas without ALT, although 80% of cases with *ATRX* mutation had ALT [62]. The notion that multiple steps are required for the development of ALT is supported by the low frequency at which it arises in immortalized cell lines, and the likely prerequisite mutations of both the G1/S and G2/M checkpoints to be permissive of the genomic instability and altered response to DNA damage seen with ALT. The G1/S checkpoint is frequently undermined by *TP53* mutation in association with *ATRX* mutation and ALT. Functional deficiencies in the G2/M checkpoint have been observed in ALT cell lines, warranting investigation of specific mutations in G2/M checkpoint signaling in ALT [19, 56].

ATRX localizes to telomeres during interphase [63], and both ATRX and DAXX are required for the deposition of H3.3 at telomeres [7]. The ATRX-DAXX complex is also involved in chromatin remodeling, and can mobilize a histone 3.3 containing nucleosome along a DNA template [7]. The consequences of disrupted H3.3 heterochromatin insertion

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

and altered chromatin remodeling are among the proposed mechanisms for the association of *ATRX* mutation and altered telomere length. Additionally ATRX is a histone code reader. The PHD domain of ATRX binds to histone 3 tails with lysine 9 tri-methylation (H3K9me3) in the absence of lysine 4 methylation, indicating that ATRX reads H3K4 and H3K9 tail marks in combination. When ATRX is unable to bind to the histone 3 tail, it fails to localize to heterochromatin [64]. Mutations affecting the histone code could potentially disrupt the association with ATRX, even in the absence of an *ATRX* mutation. ATRX has also been shown to bind GC-rich tandem sequences of telomeres and euchromatin, with mutation of *ATRX* leading to dysregulation of GC-rich tandem repeat associated genes [65]. Therefore *ATRX* mutation could indirectly affect telomere length by altering expression of telomere homologous recombination regulatory elements [19].

Histone methyltransferase or DNA methyltransferase knockout in mouse cell lines results in increased telomere length, suggesting that telomere length can be influenced by either altered histone methylation or altered DNA methylation [63, 66, 67]. This is intriguing since *ATRX* mutations and histone H3 gene mutations are independently associated with altered methylation of subtelomeric regions [68] and chromosome ends [30, 32]. Subtelomeric methylation changes could potentially be explained by similarity of ATRX's PHD domain to the DNMT3 (DNA methyltransferase 3) family of DNA methyltransferases [63, 68], and cells deficient specifically in DNMT3 also have increased telomere length [67].

Additionally, loss of ATRX is associated with abnormal retention of the telomere RNA transcript (TERRA) and replication protein A (RPA) at telomere ends after completion of DNA replication. This leads to prolonged recruitment of the protein kinase ATR (ataxia-telangiectasia mutated and Rad3 related) to telomere ends, a regulator of homologous recombination, promoting an environment for recombination outside of normal regulatory mechanisms [18].

Ultimately a downstream consequence of *ATRX* mutation, when combined with other mutations or epigenetic changes, is highly likely to create an altered telomere chromatin environment that is more permissive of homologous recombination and resultant ALT. Lack of insertion of histone 3.3, changes to DNA and telomere methylation from *ATRX* mutation or histone H3 mutation, altered expression of homologous recombination regulatory elements, and retention of TERRA with prolonged recruitment of ATR are all currently proposed mechanisms for the development of ALT.

Therapeutic interventions targeting the histone code and telomere length in gliomas

Understanding the role of histone mutations and alterations of the histone code in gliomagenesis has led to the development of potentially targeted therapeutic interventions. In particular GSKJ4, a small molecule inhibitor of the histone H3 K27 demethylase JMJD3, resulted in decreased tumor cell viability and increased H3K27me3 levels in K27M glioma cell lines, and significantly extended survival of mice with K27M mutant glioma xenografts. In contrast, GSKJ4 has not shown activity in an *H3F3A* G34V mutant glioma cell line [69]. Panobinostat, a histone deacetylase inhibitor, resulted in decreased tumor cell viability in

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

both K27M mutant glioma cell lines and in mice with K27M mutant glioma xenografts. Panobinostat treatment demonstrated a dose dependent increase in histone acetylation and in H3K27me3, which may be explained by reduced PRC2 inhibition secondary to increased histone tail acetylation [70, 71]. Combined use of GSKJ4 and panobinostat produced a synergistic reduction of tumor cell viability in K27M mutant glioma cell lines [70].

Cancer cell lines with decreased H3K36me3 secondary to inactivating *SETD2* mutation, silencing, or knockout are sensitive to WEE1 kinase inhibition [72], creating the possibility of targeted intervention in diffuse gliomas with *SETD2* or G34R/V mutations. WEE1 inhibition induces degradation of RRM2, a ribonucleotide reductase subunit involved in the formation of deoxyribonucleotides from ribonucleotides. H3K36 trimethylation normally induces transcription of RRM2, and reduced H3K36 methylation causes decreased levels of RRM2. In the setting of reduced H3K36 methylation, the effect of WEE1 inhibition on RRM2 degradation exacerbates already reduced RRM2 levels reaching a critical level of dNTP depletion that inhibits DNA replication and induces apoptosis [72].

Additionally, gliomas with G34R/V mutations have been shown to upregulate MYCN, through altered genomic binding of the histone 3 tail at K36 [75]. Therefore PI3K/mTOR inhibitors such as NVP-BEZ235, which result in MYCN degradation [76], may potentially be therapeutic in G34R/V gliomas.

Inhibitors against mechanisms of telomere maintenance are in development. The telomerase inhibitor GRN163L (Imetelstat) is an oligonucleotide complementary to the telomerase RNA template, and has entered Phase II clinical trials for pediatric recurrent or refractory brain tumors [73, ClinicalTrials.gov NCT01836549]. Additionally inhibitors of the protein kinase ATR, a regulator of homologous recombination with prolonged recruitment to telomere ends in the setting of ATRX mutation, have been found to selectively induce death of cancer cells with ALT [18].

SUMMARY

Pediatric and adult gliomas contain genetic alterations that affect the histone code, most frequently seen by direct mutation of the histone H3 tail in pediatric gliomas and *IDH1/2* mutation in adult gliomas. Histone H3 tail mutations are mutually exclusive with *IDH1/2* mutations and both uniquely alter histone tail methylation. The K27M and G34R/V mutations are associated with decreased histone tail methylation and DNA hypomethylation, while *IDH1/2* mutant gliomas correlate with DNA hypermethylation and 2-HG mediated inhibition of histone demethylation. The prevalence of *IDH1/2* or histone H3 mutations within diffuse gliomas emphasizes the delicate balance of histone methylation and the functional importance of the histone code in gliomagenesis of both adults and children.

Mutations affecting telomere length are also distinctly different in pediatric and adult gliomas. *TERT* promoter mutations are frequently seen in adult primary glioblastomas and oligodendrogliomas but are extremely rare in pediatric gliomas. In contrast to the high frequency of *ATRX* mutations in adult lower-grade astrocytomas, *ATRX* mutations are not prevalent in pediatric low-grade gliomas. While some mechanism of telomere maintenance

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

has been proposed as a requirement for gliomagenesis in adult gliomas, it does not appear to be necessary in pediatric low-grade gliomas but does contribute to pediatric high-grade gliomas. In pediatric high-grade gliomas, ALT and increased TERT expression associated with promoter hypermethylation have been observed.

Understanding differences in pediatric and adult diffuse gliomas may ultimately allow for increased prognostic precision and targeted intervention. Current therapies in development include WEE-1 inhibition for gliomas with *SETD2* or G34R/V mutation, demethylase and deacetylase inhibitors for K27M mutant gliomas, PI3K/mTOR inhibitors for G34R/V mutant gliomas, telomerase inhibitors for gliomas with *TERT* promoter mutation, and ATR inhibitors for gliomas with ALT.

Acknowledgments

We thank Rishi Bhatnagar for creation of the illustration in Figure 1. D.A.S. is supported by NIH Director's Early Independence Award (DP5 OD021403) and Career Development Award from the UCSF Brain Tumor SPORE (P50 CA097257).

References

1. Schwartzentruber J, et al. Driver mutations in histone H3.3 and chromatin remodeling genes in pediatric glioblastoma. Nature. 2012; 482:226–231. [PubMed: 22286061]
2. Chi P, Allis CD, Wand GG. Covalent histone modifications – miswritten, misinterpreted and mis-erased in human cancers. Nature Reviews Cancer. 2010; 10:457–69. [PubMed: 20574448]
3. Buczkowicz P, et al. Pathology, Molecular genetics, and epigenetics of diffuse intrinsic pontine glioma. Front Oncol. 2015; 5:1–9. [PubMed: 25667919]
4. Albig, et al. The human replacement histone H3.3B gene (H3F3B). Genomics. 1995; 30:264–72. [PubMed: 8586426]
5. Szender E, Ray-Gallet D, Almouzni G. The double face of the histone variant H3.3. Cell Research. 2011; 21:421–434. [PubMed: 21263457]
6. Kallappagoudar S, et al. Histone H3 mutations – a special role for H3.3 in tumorigenesis? Chromosoma. 2015; 124:177–189. [PubMed: 25773741]
7. Lewis P, et al. Daxx is an H3.3 specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres. Proc Natl Acad Sci USA. 2010; 107:14075–14080. [PubMed: 20651253]
8. Goldberg AD, et al. Distinct factors control histone variant H3.3 localization at specific genomic regions. Cell. 2010; 140:678–691. [PubMed: 20211137]
9. Drane P, et al. The death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3.3. Genes Dev. 2010; 24:1253–1265. [PubMed: 20504901]
10. Blackburn EH, et al. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. Science. 2015; 350:1193–1198. [PubMed: 26785477]
11. Giardini M, et al. Telomere and telomerase biology. Prog Mol Biol Transl Sci. 2014; 125:1–40. [PubMed: 24993696]
12. Conomos D, et al. Alternative lengthening of telomeres: remodeling the telomere architecture. Front Oncol. 2013; 3:27. [PubMed: 23429284]
13. Kim N, et al. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994; 266:2011–5. [PubMed: 7605428]
14. Killela P, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci USA. 2013; 110:6021–6. [PubMed: 23530248]
15. Huang FW, et al. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013; 339:957–959. [PubMed: 23348506]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

16. Horn S, et al. TERT promoter mutations in familial and sporadic melanoma. Science. 2013; 339:959–961. [PubMed: 23348503]
17. Bell RJ, et al. The transcription factor GABP selectively binds and activates the mutant TERT promoter in cancer. Science. 2015; 348:1036–9. [PubMed: 25977370]
18. Flynn R, et al. Alternative lengthening of telomeres renders cancer cells hypersensitive to ATR inhibitors. Science. 2015; 347:273–277. [PubMed: 25593184]
19. Lovejoy C, et al. Loss of ATRX, genome instability, and an altered DNA damage response are hallmarks of the alternative lengthening of telomeres pathway. PLoS Genet. 2012; 8:e1002772. [PubMed: 22829774]
20. Heaphy C, et al. Altered telomeres in tumors with ATRX and DAXX mutations. Science. 2011; 333:425. [PubMed: 21719641]
21. He LJ, et al. Prognostic significance of overexpression of EZH2 and H3k27me3 proteins in gastric cancer. Asian Pac J Cancer Prev. 2012; 13:3173–3178. [PubMed: 22994729]
22. Bae WK, et al. The methyltransferase EZH2 is not required for mammary cancer development, although high EZH2 and low H3K27me3 correlate with poor prognosis of ER-positive breast cancers. Mol Carcinog. 2015; 54:1172–1180. [PubMed: 25043748]
23. He WP, et al. Decreased expression of H3K27me3 in human ovarian carcinomas correlates with more aggressive tumor behavior and poor patient survival. Neoplasma. 2015; 62:932–937. [PubMed: 26458314]
24. Wei Y, et al. Loss of trimethylation at lysine 27 of histone H3 is a predictor of poor outcome in breast, ovarian, and pancreatic cancers. Mol Carcinog. 2008; 47:701–706. [PubMed: 18176935]
25. Ngollo M, et al. The association between histone 3 lysine 27 trimethylation (H3K27me3) and prostate cancer: relationship with clinicopathological parameters. BMC Cancer. 2014; 14:994. [PubMed: 25535400]
26. Oh EJ, et al. Diffuse large B-cell lymphoma with histone H3 trimethylation at lysine 27: another poor prognostic phenotype independent of c-Myc/Bcl2 coexpression. Hum Pathol. 2014; 45:2043–2050. [PubMed: 25149548]
27. Ntziachristos, et al. Contrasting roles for histone 3 lysine 27 demethylases in acute lymphoblastic leukemia. Nature. 2014; 514:513–517. [PubMed: 25132549]
28. Khuong-Quang DA, et al. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. Acta Neuropathol. 2012; 124:439–447. [PubMed: 22661320]
29. Wu G, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. Nat Genet. 2012; 44:251–253. [PubMed: 22286216]
30. Sturm D, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Cancer Cell. 2012; 4:425–437.
31. Behjati S, et al. Distinct H3F3A and H3F3B driver mutations define chondroblastoma and giant cell tumor of bone. Nat Genet. 2013; 45:1479–82. [PubMed: 24162739]
32. Bender S, et al. Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. Cancer Cell. 2013; 24:660–672. [PubMed: 24183680]
33. Solomon D, et al. Diffuse midline gliomas with histone H3-K27M mutation: a series of 47 cases assessing the spectrum of morphologic variation and associated genetic alterations. Brain Pathol. 2016; 26:569–80. [PubMed: 26517431]
34. Gessi M, et al. High frequency of H3F3A (K27M) mutations characterizes pediatric and adult high-grade gliomas of the spinal cord. Acta Neuropathol. 2015; 130:435–437. [PubMed: 26231952]
35. Feng J, et al. The H3.3 K27M mutation results in a poorer prognosis in brainstem gliomas than thalamic glioms in adults. Human Pathol. 2015; 46:1626–1632. [PubMed: 26297251]
36. Buczkowicz P, et al. Histopathological spectrum of paediatric diffuse intrinsic pontine glioma: diagnostic and therapeutic implications. Acta Neuropathol. 2014; 128:573–581. [PubMed: 25047029]
37. Pathak P, et al. Altered global histone-trimethylation code and H3F3A-ATRX mutation in pediatric GBM. J Neurooncol. 2015; 121:489–497. [PubMed: 25479829]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

38. Fontebasso AM, et al. Mutations in SETD2 and genes affecting histone H3K36 methylation target hemispheric high-grade gliomas. Acta Neuropathol. 2013; 125:659–669. [PubMed: 23417712]
39. Lewis PW, et al. Inhibition of PRC2 activity by a gain-of function H3 mutation found in pediatric glioblastoma. Science. 2013; 340:857–861. [PubMed: 23539183]
40. Hirabayashi Y, Suzki N, Tsuboi M, et al. Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition. Neuron. 2009; 63:600–613. [PubMed: 19755104]
41. Xu W, et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. Cancer Cell. 2011; 19:17–30. [PubMed: 21251613]
42. Chowdhury R, et al. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. EMBO Rep. 2011; 12:463–469. [PubMed: 21460794]
43. Lu C, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature. 2012; 483:474–480. [PubMed: 22343901]
44. Arita H, et al. Upregulating mutations in the TERT promoter commonly occur in adult malignant gliomas and are strongly associated with total 1p19q loss. Acta Neuropathol. 2013; 126:267–276. [PubMed: 23764841]
45. Koelsche C, et al. Distribution of TERT promoter mutations in pediatric and adult tumors of the nervous system. Acta Neuropathol. 2013; 126:907–915. [PubMed: 24154961]
46. Eckel-Passow JE, et al. Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. N Engl J Med. 2015; 372:2499–508. [PubMed: 26061753]
47. The Cancer Genome Atlas Research Network. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med. 2015; 372:2481–2497. [PubMed: 26061751]
48. Nonoguchi N, et al. TERT promoter mutations in primary and secondary glioblastomas. Acta Neuropathol. 2013; 126:931–937. [PubMed: 23955565]
49. Labussiere M, et al. Combined analysis of TERT, EGFR, and IDH status defines distinct prognostic glioblastomas classes. Neurology. 2014; 83:1200–6. [PubMed: 25150284]
50. Castelo-Branco P, et al. Methylation of the TERT promoter and risk stratification of childhood brain tumours: an integrative genomic and molecular study. Lancer Oncol. 2013; 14:534–42.
51. Guilleret I, et al. Hypermethylation of the human telomerase catalytic subunit (hTERT) gene correlates with telomerase activity. Int J Cancer. 2002; 101:335–341. [PubMed: 12209957]
52. Lindsey JC, et al. TERT promoter mutation and aberrant hypermethylation are associated with elevated expression in medulloblastoma and characterize the majority of non-infant SHH subgroup tumours. Acta Neuropathol. 2014; 127:307–309. [PubMed: 24337442]
53. Fan Y, et al. Telomerase expression by aberrant methylation of the TERT promoter in melanoma arising in giant congenital nevi. J Invest Dermatol. 2016; 136:339–342. [PubMed: 26763461]
54. Arita H, et al. TERT promoter mutations rather than methylation are the main mechanism for TERT upregulation in adult gliomas. Acta Neuropathol. 2013; 126:939–941. [PubMed: 24174165]
55. Jiao Y, et al. DAXX/ATRX, MEN1 and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. Science. 2011; 331:1199–1203. [PubMed: 21252315]
56. Mangerel J, et al. Alternative lengthening of telomeres is enriched in, and impacts survival of TP53 mutant pediatric malignant brain tumors. Acta Neuropathol. 2014; 128:853–862. [PubMed: 25315281]
57. Liu X, et al. Frequent ATRX mutations and loss of expression in adult diffuse astrocytic tumors carrying IDH1/IDH2 and TP53 mutations. Acta Neuropathol. 2012; 124:615–625. [PubMed: 22886134]
58. Zhang J, et al. Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. Nat Genet. 2013; 45:602–12. [PubMed: 23583981]
59. Tabori U, et al. The role of telomere maintenance in the spontaneous growth arrest of pediatric low-grade gliomas. Neoplasia. 2006; 8:136–142. [PubMed: 16611406]
60. Jiao Y, et al. Frequent ATRX, CIC, FUBP1 and IDH1 mutations refine the classification of malignant gliomas. Oncotarget. 2012; 3:709–22. [PubMed: 22869205]
61. Suzuki H, et al. Mutational landscape and clonal architecture in grade II and III gliomas. Nat Genet. 2014; 47:458–68.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

62. Abedalthagafi M, et al. The alternative lengthening of telomere phenotype is significantly associated with loss of ATRX expression in high-grade pediatric and adult astrocytomas: a multi-institutional study of 214 astrocytomas. Mod Pathol. 2013; 26:1425–1432. [PubMed: 23765250]
63. Wong LH, et al. ATRX interacts with H3.3 in maintaining telomere structural integrity in pluripotent embryonic stem cells. Genome Res. 2010; 20:351–60. [PubMed: 20110566]
64. Dhayalan A, et al. The ATRX-ADD domain binds to H3 tail peptides and reads the combined methylation state of K4 and K9. Hum Mol Genet. 2011; 20:2195–2203. [PubMed: 21421568]
65. Law MJ, et al. ATR-X syndrome protein targets tandem repeats and influences allele-specific expression in a size dependent manner. Cell. 2010; 143:367–378. [PubMed: 21029860]
66. Garcia-Cao M, et al. Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases. Nat Genet. 2004; 36:94–9. [PubMed: 14702045]
67. Gonzalo S, et al. DNA methyltransferases control telomere length and telomere recombination in mammalian cells. Nat Cell Biol. 2006; 8:416–24. [PubMed: 16565708]
68. Gibbons RJ, et al. Mutations in ATRX, encoding a SWI/SNF-like protein, cause diverse changes in the pattern of DNA methylation. Nat Genet. 2000; 24:368–71. [PubMed: 10742099]
69. Hashizume R, et al. Pharmacologic inhibition of histone demethylation as a therapy for pediatric brainstem glioma. Nat Med. 2014; 20:1394–1396. [PubMed: 25401693]
70. Grasso CS, et al. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. Nat Med. 2015; 21:555–559. [PubMed: 25939062]
71. Brown ZZ, et al. Strategy for “detoxification” of a cancer-derived histone mutant based on mapping its interaction with the methyltransferase PRC2. J Am Chem Soc. 2014; 136:13498–13501. [PubMed: 25180930]
72. Pfister SX, et al. Inhibiting WEE1 selectively kills histone H3K36me3-deficient cancers by dNTP starvation. Cancer Cell. 2015; 28:557–568. [PubMed: 26602815]
73. Ruden M, Puri N. Novel anticancer therapeutics targeting telomerase. Cancer Treatment Rev. 2013; 39:444–56.
74. Lee W, et al. PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. Nat Genet. 2014; 46:1227–32. [PubMed: 25240281]
75. Bjerke L, et al. Histone H3.3. mutations drive pediatric glioblastoma through upregulation of MYCN. Cancer Discov. 2013; 3:512–519. [PubMed: 23539269]
76. Vaughan L, et al. Inhibition of mTOR-kinase destabilizes MYCN and is a potential therapy for MYCN-dependent tumors. Oncotarget. 2016 Epub 2016 Jul 12.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

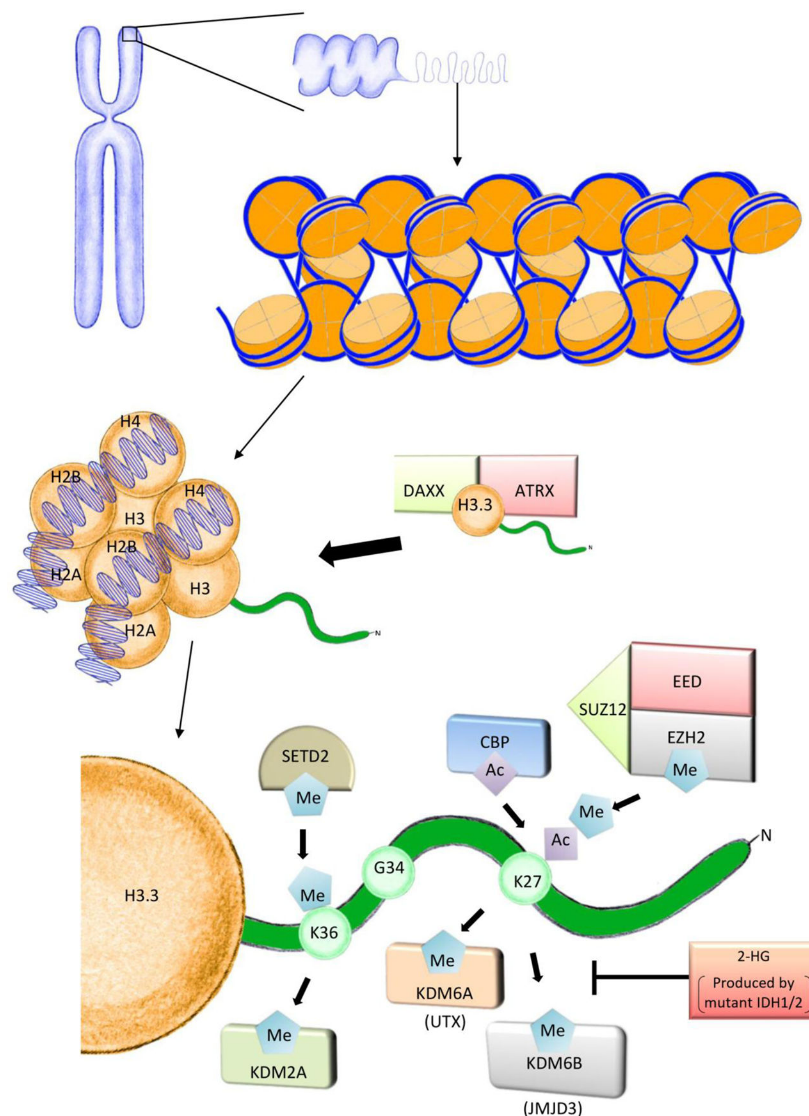

Figure 1.

Illustration of the histone code that is disrupted by genetic alterations in pediatric and adult gliomas. The histone octamer is depicted as the protein core of the nucleosome, in relation to overall chromatin structure (top panel). The deposition of the histone variant H3.3 into nucleosomes at telomeres requires the ATRX-DAXX chromatin remodeling complex (middle panel). The N-terminal histone tail of H3.3 is shown (bottom panel), with emphasis on the K27 and G34 amino acid residues that are frequently mutated in diffuse gliomas. The K36 residue is also shown as it is a critical site of post-translational modification, affected by both G34R/V mutations and mutations in the K36 methyltransferase SETD2. Writers and erasers of the histone code are displayed including the enhancer of zeste homolog 2 (EZH2), the K27 methyltransferase catalytic subunit of the polycomb repressive complex 2 which is inhibited by K27M H3.3 mutation. EED and SUZ12 are other members of the polycomb repressive complex 2; while they are not commonly mutated in diffuse gliomas they are involved in tumorgenesis of other neoplasms such as malignant peripheral nerve sheath

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

tumors [74]. Also shown is JMJD3, the K27 demethylase that is one potential target of therapeutic intervention for K27M mutant gliomas. The oncometabolite 2-HG (2-hydroxyglutarate), produced by mutant IDH1/2 enzymes, is shown inhibiting H3K27 demethylation. Other key histone acetyltransferases (CBP) and demethylases (KDM2A and UTX) are shown to demonstrate the complexity of histone code regulation at amino acid residues K27 and K36, although these enzymes are not frequently mutated in diffuse gliomas.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

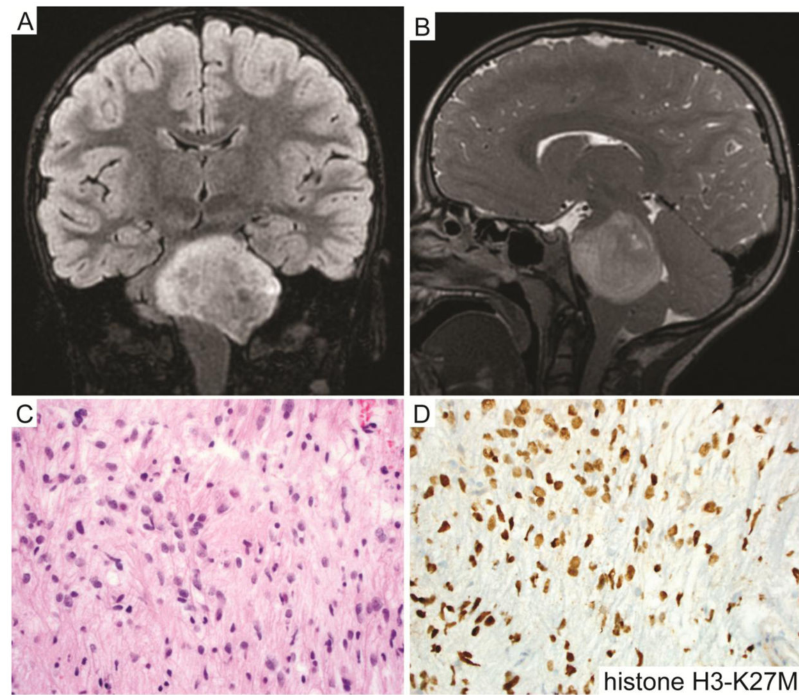

Figure 2.

Example of a diffuse intrinsic pontine glioma with histone H3 K27M mutation in a child. **A, B.** Coronal T2-weighted fluid-attenuated inversion recovery (A) and sagittal T2-weighted (B) MR images demonstrating an expansile mass centered in the pons. **C.** H&E stained section showing an infiltrative astrocytoma. **D.** Immunohistochemical stain for histone H3 K27M mutant protein showing strong nuclear positivity in tumor cells.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

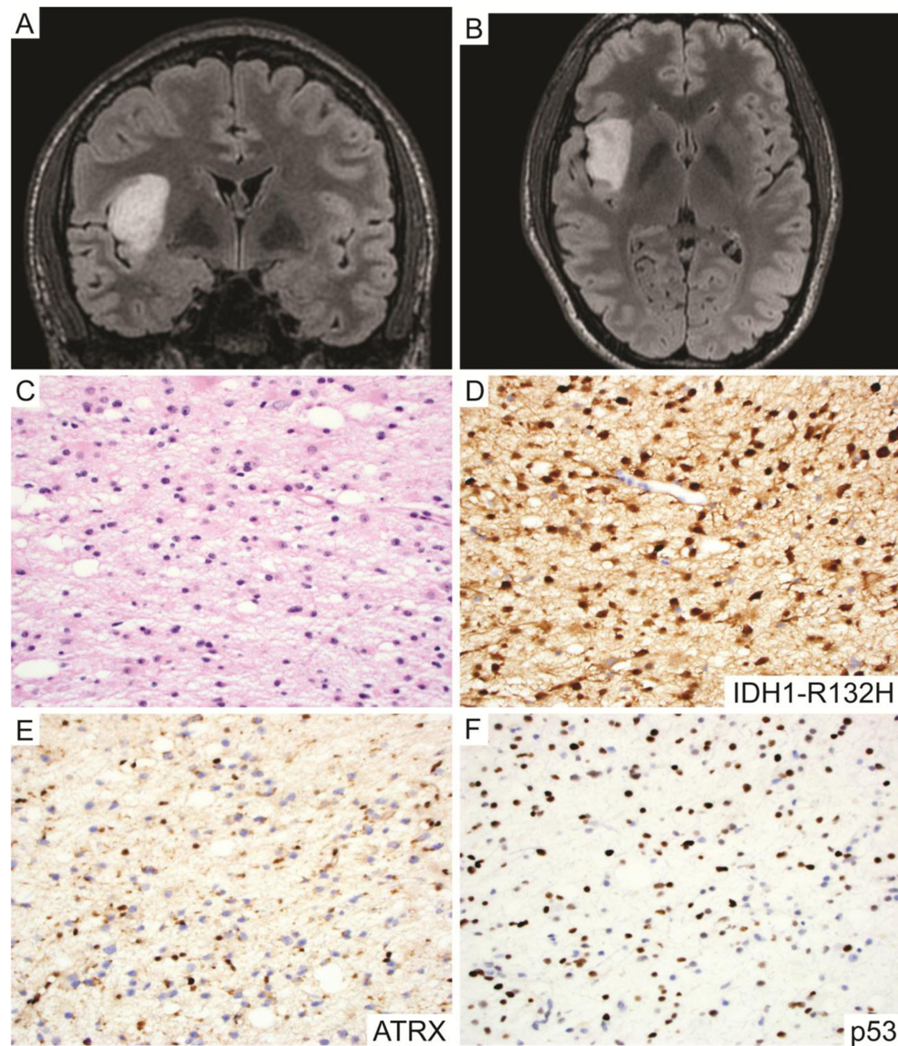

Figure 3.
Example of a diffuse astrocytoma arising in the cerebral hemisphere of a young adult with IDH1 R132H mutation, ATRX loss, and p53 overexpression. **A, B.** Coronal (A) and axial (B) T2-weighted fluid-attenuated inversion recovery MR images demonstrating an expansile mass in the right insula. **C.** H&E stained section showing an infiltrating astrocytoma. **D.** Immunohistochemical stain for IDH1 R132H mutant protein showing strong positivity in tumor cells. **E.** Immunohistochemical stain for ATRX showing absence of staining in tumor cells with retained expression in endothelial cells and entrapped neurons. **F.** Immunohistochemical stain for p53 showing strong nuclear positivity in the majority of tumor cells.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Frequency and prognosis of histone and telomere gene alterations in pediatric and adult diffuse gliomas.

GENETIC ALTERATIONS	TUMOR HISTOLOGIC TYPE	TUMOR LOCATION	AGE GROUP	APPROXIMATE FREQUENCY	PROGNOSIS
H3 K27M	Diffuse midline glioma	Midline structures including thalamus, brainstem, and spinal cord	Pediatric	80% in pontine tumors 50–80% in thalamic and spinal cord tumors	Very poor prognosis irrespective of histologic grade
			Adult	50–60%	Poor prognosis except possibly in thalamic gliomas
H3 G34R/V	High grade diffuse astrocytoma	Cerebral hemispheres	Pediatric	20–30%	Associated with better prognosis than H3 K27M mutant diffuse gliomas
SETD2	High grade diffuse astrocytoma	Cerebral hemispheres	Pediatric	15%	Unknown
			Adult	Rare	Unknown
TERT promoter	Oligodendroglioma	Cerebral hemispheres	Adult	>95%	Better prognosis compared to age-matched diffuse lower-grade gliomas with ATRX mutation
	Primary glioblastoma	Cerebral hemispheres	Adult	80%	Worse prognosis than TERT promoter wild-type primary glioblastomas
ATRX	High grade diffuse astrocytoma	Cerebral hemispheres	Pediatric	30–40%	Unknown
	Diffuse midline glioma	Midline structures including thalamus, brainstem, and spinal cord	Pediatric	10% in pontine tumors 30–50% in thalamic and spinal cord tumors	Unknown
			Adult	50–70%	Unknown
	Lower-grade diffuse astrocytomas and secondary glioblastomas	Cerebral hemispheres	Adult	>90%	Worse prognosis compared to age-matched diffuse lower-grade gliomas with TERT promoter mutation