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## Histone H3 Variants in the Multiverse of Cancer

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*Annual Review of Cancer Biology*Histone H3 Variants in the  
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**Keywords**

oncohistones, H3 variants, chromatin reprogramming, cancer cell of origin, ALT, alternative lengthening of telomeres, EMT, epithelial-to-mesenchymal transition, radioresistance

**Abstract**

Our understanding of cancer genomes has allowed for the integration of molecular data into histopathological classifications for routine stratification of patients. In the last 10–15 years, thanks to this systematic implementation of large-scale sequencing, the identification of hotspot somatic mutations in histone genes came into the limelight, underscoring the concept of oncohistones. As drivers in pediatric brain tumors, and in several other types of cancers, oncohistones brought a “new dimension of Strange” into the cancer multiverse, to paraphrase Marvel. An integrative approach to cancer complexity as a multidimensional projection is urgently needed to consider all relevant etiological, developmental, and evolutionary components. Here, we discuss recent progress on histone variants and chaperones, their regulation and alterations in cancers, the available *in vivo* models, and current treatment strategies. More specifically, we adopt a view through the lens of tissue-specific differences and means for genome expression and integrity maintenance.

## 1. INTRODUCTION

All genome functions in eukaryotes operate in a chromatin context within the cell nucleus, where DNA is organized in a nucleoprotein complex with histones, the most abundant protein component (Yadav et al. 2018). Albrecht Kossel (1928), a Nobel laureate in physiology and medicine who determined the biochemical composition of nucleic acids, was the first to isolate the histones. Chromatin organization is ancestral to the appearance of the nucleus in eukaryotes, and histones are already present in archaea (Mattioli et al. 2017), some viruses (Liu et al. 2021, Talbert et al. 2022), and, in specific situations, some prokaryotes (Rojec et al. 2019, Hocher et al. 2023). This realization further underlines the fundamental role of histones in chromatin, the scaffold for genome transactions and integrity maintenance in the tree of life.

The nucleosome is the basic unit of chromatin. Its crystal structure, resolved at 2.8 Å (Luger et al. 1997), showed in detail how histone heterodimers formed, via the globular histone fold domain, in both H3-H4 and H2A-H2B heterodimers in a handshake model. Approximately 147 base pairs of DNA are wrapped around this histone octamer. In addition, at the exit of the nucleosome core particle associated with linker DNA, members of the histone H1 family stabilize the nucleofilament and thereby contribute to higher-order chromatin folding. The core histone H3-H3 interface, conserved between eukaryotes and archaea, carries an ancestral copper reductase function (Attar et al. 2020). This finding implies a role for H3 in genome protection mechanisms already in the early eukaryotes by facilitating cellular copper usage in the challenging oxidizing conditions of an oxygenated atmosphere (Rudolph & Luger 2020). A functional and regulatory complexity to the diversification of histones in eukaryotes is added by the unstructured N-terminal histone tails, which are not found in archaea. In this context, the discovery of enzymes, imposing a myriad of covalent posttranslational modifications (PTMs) on histone tails and, to a lesser extent, in the fold domain, led to much excitement regarding the possibility of regulating genome information (Jenuwein & Allis 2001, Audia & Campbell 2016, Millán-Zambrano et al. 2022). Cross talk between PTMs and their combination can affect both chromatin properties and enzymes functioning on chromatin (Suganuma & Workman 2008). Moreover, histone PTMs may favor the interaction of different binding proteins, affect internucleosome interactions, and influence chromatin structure and accessibility. In this way, PTMs contribute to the regulation of several DNA metabolism processes, such as replication, transcription, repair, and chromosome segregation (Bannister & Kouzarides 2011). Notably, hotspot mutations in H3 histone genes, first reported to occur in cancers, affect precise key amino acids in the N-terminal region whose PTMs exert regulatory functions. These somatic missense mutations correspond to K27M or G34R/V substitutions in a subset of brain high-grade glioma, such as diffuse intrinsic pontine glioma (DIPG) and hemispheric pediatric high-grade glioma (pHGG) (Schwartzentruber et al. 2012, Wu et al. 2012), while somatic missense mutations corresponding to G34W/L or K36M substitutions characterize tumors of the long bones (Behjati et al. 2013). Thus, given that a series of cancers harbor oncohistones, histone variants and PTM changes have gained a lot of attention. H3K4me, H3K9me, H3K27me, and H3K36me, which are among the most studied lysine methylation sites on histone H3, are all affected by these hotspot mutations (Bjerke et al. 2013, Lewis et al. 2013, Lu et al. 2016, Voon et al. 2018, Chaouch et al. 2021, Furth et al. 2022). H3K27 is a key residue posttranslationally modified by the Polycomb complexes, and the H3K27M mutants lead to a dominant-negative alteration of H3K27me<sub>3</sub> levels genome wide (Lewis et al. 2013, Harutyunyan et al. 2019). The epigenomic changes in H3.3G34R-harboring tumors vary across cell types. In the context of a H3.3G34R substitution, a loss of H3K9me<sub>3</sub>/H3K36me<sub>3</sub> occurred in mouse embryonic stem cells (Voon et al. 2018) and also in patient-derived cell lines (C.C.L. Chen et al. 2020). However, in human fetal neural progenitors this mutation did not drive major changes in

active H3K4me3/H3K36me3 or repressive H3K27me3 marks but rather reinforced preexisting progenitor transcriptional programs (Bressan et al. 2021). Finally, the H3K36M mutation led to a decrease in H3K36me2/H3K36me3 marks and an increase in H3K27me3, with a dominant effect on wild-type H3 PTMs (Lu et al. 2016). In all situations, oncohistones impaired normal developmental circuits and promoted the expansion of specific progenitor cells at the expense of differentiation (Deshmukh et al. 2022).

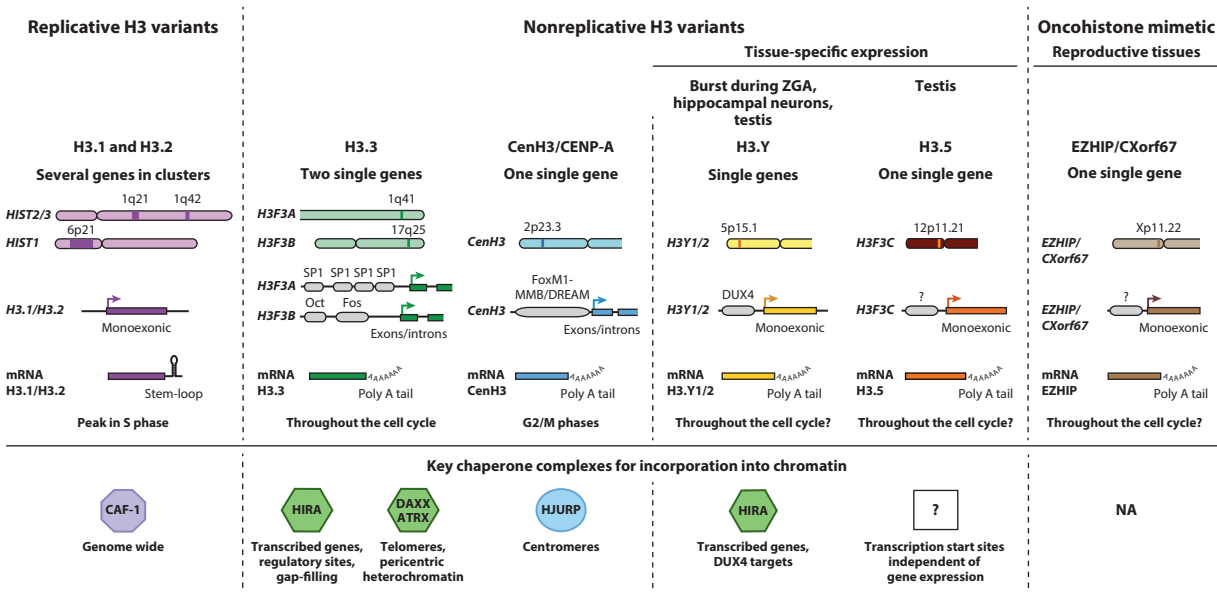
Chromatin remodeling, along with H3K9 and H3K27 methylation changes, also helps secure genome integrity possibly by interfering with nuclear stiffness and calcium signaling (Nava et al. 2020). The previous link between a H3.3K27M oncohistone and perturbed calcium signaling (Delaney et al. 2019) may also exploit a reduction of nuclear stiffness by affecting heterochromatin in DIPG (Deng et al. 2016). Furthermore, the H3.3G34R oncohistone with its dominant-negative effect on H3K9me3 also emphasizes the effect on heterochromatin (Voon et al. 2018) and leads to increased DNA damage (Haase et al. 2022). In both cases, the link to nuclear stiffness remains to be explored. Thus, oncohistone variants, beyond their implication in transcriptional regulation changes, may participate in shaping the mechanosensory and physical properties of “oncochromatin,” an underappreciated aspect of the effects of oncohistones.

## **2. HISTONE H3 VARIANTS: GENOMIC ORGANIZATION, EXPRESSION, DEPOSITION PATHWAYS, AND DEREGULATION IN CANCERS**

Eukaryotic histones exist as variants and are escorted by distinct histone chaperones during cellular traffic. Their incorporation into distinct chromosomal regions defines chromatin landscapes typical of different cell types. Yet the plasticity of this organization enables cellular transition, a versatile system important for proper organ formation and function (Yadav et al. 2018). Histone variants are split into two groups. The replicative variants, whose expression peaks in S phase, represent most histones in proliferating cells and are used in concert with the replicative machinery for a deposition coupled to DNA synthesis. The nonreplicative histones, whose expression is not tightly associated with DNA synthesis, are present in both dividing and nondividing cells and are expressed in a temporal and tissue-specific manner. They display distinct enrichment at chromosomal landmarks characterized by different structural and functional properties, such as telomeres, centromeres, and heterochromatin domains. The histone H3 family comprises several variants with associated chaperones (Ray-Gallet & Almouzni 2021). Here, we detail those variants associated with cancer, as depicted in **Figure 1**.

### **2.1. H3.1 and H3.2**

In mammals, the replicative H3.1 and H3.2 histones are 136 amino acids long, with one residue difference, cysteine versus serine at position 96 (reviewed in Szenker et al. 2011). H3.1 and H3.2, found in complex with the histone chaperone complex chromatin assembly factor 1 (CAF-1) (Tagami et al. 2004), are deposited broadly in a DNA synthesis-coupled manner connected to the replication machinery (Delaney et al. 2023). Three gene clusters encode H3.1 and H3.2, as well as H2A, H2B, and the H1 linker, and comprise more than 60 copies of histone genes, forming the histone locus bodies (reviewed in Marzluft & Koreski 2017, Mendiratta et al. 2019) (**Figure 1**). Transcripts corresponding to replicative histones, including H3.1 and H3.2, exhibit a peculiar architecture, as their maturation, stability, and translation are regulated by a 3' stem-loop rather than by polyadenylation (poly A) signals. Thus, since most transcriptomic analyses to date have used poly A detection, changes in replicative histone transcripts could have been missed. Curiously, under stress conditions often linked with cellular transformation, H3.1 can be



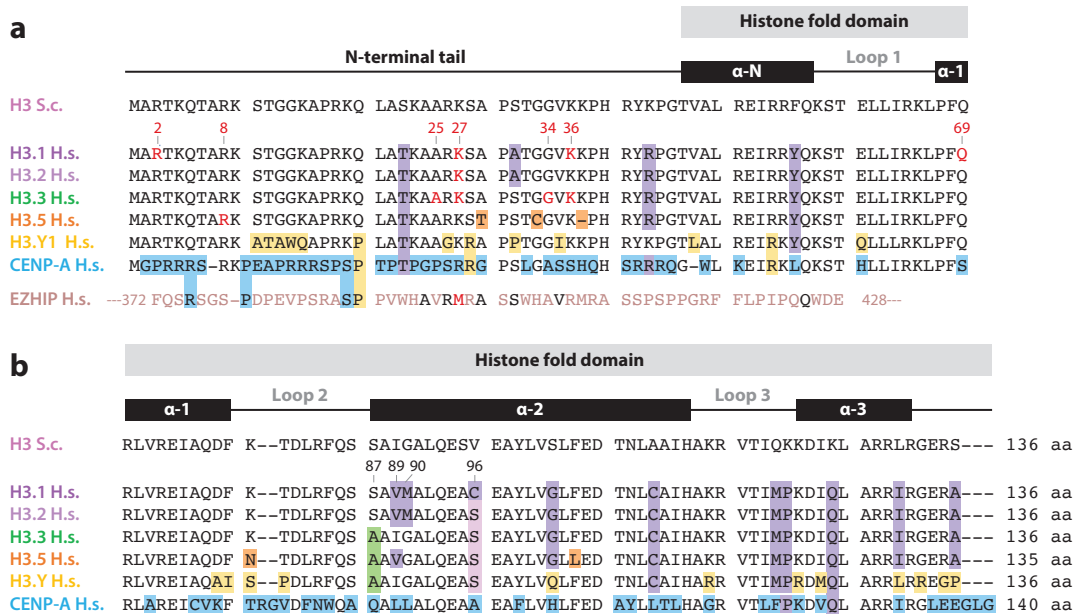
**Figure 1**

Histone H3 variants deregulated in cancers: genomic organization, mRNA structure, temporal expression in normal situations, and key chaperone complexes for incorporation into chromatin. Replicative histones H3.1 and H3.2 are expressed from 15 intron-less genes organized into three clusters. Their expression peaks in S phase and their transcripts are regulated by a 3' stem-loop. Two single genes containing introns encode H3.3 proteins with identical amino acid composition, expressed throughout the cell cycle. Differences in the transcription factor binding sites account for specific regulation of H3.3 gene products. A single gene encodes CENPA, which is expressed in G2/M phases. The shared FoxM1-MuvB/DREAM complex-binding motif ensures either CENPA transactivation or transcriptional repression. The tissue-specific variants H3.Y1, H3.Y2, and H3.5 are encoded by single H3.3 pseudogene-derived intron-less genes. In normal tissues, H3.Y1 and H3.Y2 transcription is regulated by the zygotic homeobox transcription factor DUX4 in a short developmental window. EZHIP is expressed from a single retrogene in reproductive tissues (testis, ovary) and placenta. H3.5 and EZHIP transcriptional regulation is not characterized extensively and is illustrated with a question mark. Chromosome locations are indicated for human. Key chaperone complexes selectively incorporate H3 variants at specific locations, contributing to local properties of chromatin (transcribed genes, regulatory sites) and chromosome territories (centromeres, telomeres). The deposition mode of H3.5 has yet to be characterized. Abbreviations: ATRX, alpha thalassemia/mental retardation syndrome X-linked; CAF-1, chromatin assembly factor 1; DAXX, death domain associated protein 6; DREAM, dimerization partner, Rb-like, E2F4/5 and MuvB; EZHIP/CXorf67, enhancer of zeste homolog inhibitory protein; HIRA, histone regulator A; HJURP, Holliday junction recognition protein; mRNA, messenger RNA; NA, not applicable; poly A, polyadenylation; ZGA, zygotic genome activation. H3.1/2/3 and CENP-A panels are adapted with permission from Ray-Gallet & Almouzni (2021).

polyadenylated (D. Chen et al. 2020). Notably, the first reports of mutations in the H3 histone genes linked them to the deadly pediatric cancers GBM and DIPG, which exhibit a heterozygous mutation in only a single allele of 1 of all 15 genes producing H3 histones (Schwartzentruber et al. 2012, Wu et al. 2012). This finding implies that H3 oncohistones operate in a dominant-negative manner, affecting the wild-type histones in *trans*, to globally change histone PTMs, chromatin and DNA metabolism, which in turn alters cellular differentiation programs (Lowe et al. 2019).

## 2.2. H3.3

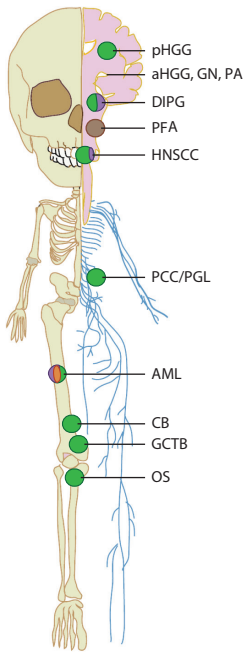
H3.3, also called a replacement variant, is ancestral to its replicative counterparts H3.1 and H3.2 (Postberg et al. 2010). H3.3 differs from H3.1 and H3.2 by five and four amino acids, respectively, yet spans the same total length (Figure 2). The differences concern a single position in the



**Figure 2**

Multiple sequence alignment of H3 variants and the conserved domain of EZHIP. (a) Sequences of human H3 variants altered in cancers are compared to yeast H3 histones, showing a strong evolutionary conservation. (b) The H3-oncohistone mimetic conserved domain of human EZHIP/CXorf67 is also shown. Amino acid residues that are mutated in cancers, as well as the EZHIP methionine homologous to the H3K27M residue, are in red and are listed in **Figure 3**. Amino acids specific to H3 variants and different from those in yeast are highlighted in colors corresponding to those used in **Figure 1**. Only H3.Y2 (also called H3.Y) is shown, as H3.Y1 (also called H3.X) was not detected at the protein level in the initial study by Wiedemann et al. (2010). The amino acids important for chaperone recognition at positions 87, 89, 90, and 96 corresponding to the H3.1 and H3.2 S-VM and the H3.3 A-IG motifs are indicated. Shown are the positions of the N-terminal tail and  $\alpha$ -helices of the histone fold domain and the protein length in amino acids. Abbreviations: aa, amino acid; EZHIP, enhancer of zeste homolog inhibitory protein.

N-terminal tail (alanine versus serine at residue 31) and a stretch in the histone fold domain, corresponding to the key region for their deposition mode into chromatin. H3.3 incorporation occurs in a DNA synthesis-independent manner (Ahmad & Henikoff 2002). The histone chaperone complex histone regulator A (HIRA), which is associated with H3.3, promotes its deposition (Tagami et al. 2004, Ricketts & Marmorstein 2017) mainly at actively transcribed regions (Goldberg et al. 2010). Another partner of H3.3, the chaperone complex death domain associated protein 6/alpha thalassemia, mental retardation syndrome X-linked (DAXX/ATRX), promotes enrichment at heterochromatin regions close to telomeres and within pericentromeres (Drané et al. 2010, Goldberg et al. 2010, Lewis et al. 2010) (**Figure 1**). At places of exposed DNA, such as regions missing H3.1 incorporation during DNA replication, an alternative HIRA-dependent H3.3 deposition could act as a gap-filling mechanism to prevent nucleosome-free DNA accumulation and to preserve chromatin stability (Ray-Gallet et al. 2011). H3.3 histones represent another conundrum, since two distinct genes, *H3F3A* and *H3F3B*, encode the exact same protein (**Figure 1**) and the two genes are subjected to nonneutral evolution and codon-usage preferences (Muhire et al. 2019). This finding is particularly intriguing, as their mutational spectra in cancers differ (**Figure 3**; Section 3.1). How the two genes may contribute to specific regulatory functions in different cellular contexts is a topic for further exploration.



Tumor location	Tumor type and frequency	H3 aa substitutions in cancers	H3 gene (or EZHIP)	References
<b>Thalamus</b>	Low-grade PA	<b>K27M</b>	No gene information, detected by IHC	Orillac et al. 2016
<b>Thalamus, spinal cord, and third ventricle</b>	Adult aHGG, GN tumors	<b>K27M</b>	No gene information, detected by IHC	Orillac et al. 2016, Solomon et al. 2016, Chia et al. 2021
<b>Brainstem, thalamus</b>	84% of pediatric DIPG 60% of high-grade nonbrainstem glioma	<b>H3.1K27M</b> or <b>H3.2K27M</b> <b>H3.3K27M</b>	<i>HIST1H3B/C</i> , <i>HIST2H3B</i> <i>H3F3A</i>	Schwartzentruber et al. 2012
<b>Brainstem</b>	Case report of DIPG	<b>H3.3K27I</b>	<i>H3F3A</i>	Castel et al. 2015
<b>Posterior fossa</b>	86% of PFA	<b>K27M-mimetic</b>	<i>EZH1P</i>	Pajtler et al. 2018, Jain et al. 2019
<b>Posterior fossa</b>	4.2% of PFA	<b>H3.3K27M</b> or <b>H3.1K27M</b>	<i>H3F3A</i> or <i>HIST1H3B/C</i>	Jain et al. 2019
<b>Brain hemispheres</b>	30% HGG	<b>H3.3G34R/V</b>	<i>H3F3A</i>	Behjati et al. 2013
<b>Head and neck</b>	HN5CCs	<b>H3.3K36M</b>	<i>H3F3B</i>	Papillon-Cavanagh et al. 2017
<b>Peripheral nervous system</b>	PCCs and PGLs	<b>H3.3G34W</b>	<i>H3F3A</i>	Toledo et al. 2016
<b>Epiphysis of long bones</b>	95% of CBs	<b>H3.3K36M</b>	<i>H3F3B</i> (rarely <i>H3F3A</i> )	Behjati et al. 2013, Fang et al. 2016
<b>Epiphysis of long bones</b>	92% of GCTBs	<b>H3.3G34W/L</b>	<i>H3F3A</i>	Behjati et al. 2013
<b>Bones</b>	6% of OS, clear-cell chondrosarcoma	<b>H3.3G34W/L</b>	<i>H3F3A</i> or <i>H3F3B</i>	Behjati et al. 2013, Koelsche et al. 2017
<b>Bone marrow</b>	AML	<b>H3.5R2Q</b> or <b>R8H</b> <b>H3.3A25P</b> <b>H3.3K27M/I</b> <b>H3.1Q69H</b>	<i>H3F3C</i> <i>H3F3A</i> <i>HIST1H3A</i>	Lehnertz et al. 2017, Boileau et al. 2019, Nacev et al. 2019

**Figure 3**

Cancer-associated mutations in H3 variants. (Left) Schematic of histone H3 alterations in different cancers. Exaggerated body parts reflect the higher tumor frequency in the corresponding locations (head and long bones). Circles indicate cancer occurrence at the indicated location. The color code used for the different H3 variants or EZHIP is the same as that used in **Figure 1**. Abbreviations: aa, amino acid; aHGG, adult high-grade glioma; AML, acute myeloid leukemia; CB, chondroblastoma; DIPG, diffuse intrinsic pontine glioma; EZHIP, enhancer of zeste homolog inhibitory protein; GCTB, giant cell tumor of bone; GN, glioneuronal tumor; HN5CC, head and neck squamous cell carcinoma; IHC, immunohistochemistry; OS, osteosarcoma; PA, pilocytic astrocytoma; PCC, pheochromocytoma; PFA, posterior fossa ependymoma A; PGL, paraganglioma; pHGG, pediatric high-grade glioma. Figure adapted from images created with BioRender.com.

### 2.3. CenH3/CENP-A

The centromeric H3 variant, called CenH3 or CENP-A, is essential to epigenetically define the centromere and to build the kinetochore in many organisms. CENP-A encodes a 140-amino-acid protein, with less than 50% identity with the three other H3 variants, from which it is the most divergent (Müller & Almouzni 2017) (**Figure 2**). This divergence is in line with the rapid centromere evolution between species (Drimmenberg et al. 2016). In mammals, CENP-A is incorporated into centromeric chromatin only in late M phase and early G1 phase, owing to its dedicated chaperone Holliday junction recognition protein (HJURP) (Dunleavy et al. 2009, Foltz et al. 2009). The forkhead homeobox protein M1 (FoxM1) in concert with the MuvB complex transactivates a myriad of G2/M cell cycle targets, including CENPA and HJURP. In contrast, the p53/p21/DREAM (dimerization partner, Rb-like, E2F4/5 and MuvB) pathway represses their transcription via specific DREAM binding sites in their promoters (**Figure 1**). This regulation correlates with the high levels of CENP-A and HJURP in cancers with an inactive p53 pathway (Filipescu et al. 2017). CENP-A overexpression in cells leads to its mislocalization in chromosome arms in a DAXX-dependent (Lacoste et al. 2014) or HIRA-dependent (Nye et al. 2018) manner. In the ectopic locations, CENP-A forms heterotypic nucleosomes with H3.3

(Lacoste et al. 2014). Importantly, CENP-A ectopic incorporation occludes certain CTCF sites, with possible implications for higher-order chromosome landscape remodeling, an aspect that deserves to be explored.

## 2.4. Tissue-Specific H3 Variants

Tissue-specific H3 variants are illustrated with H3.Y1, H3.Y2 (Wiedemann et al. 2010), and H3.5 (Schenk et al. 2011), all of which are encoded by single intron-less genes evolutionarily related to H3.3 (**Figure 1**). H3.Y1 and H3.Y2 are situated in the TAF11-like microsatellite repeat region (Resnick et al. 2019) and are transcribed in cell lines and most tissues (Wiedemann et al. 2010). They are transactivated by the zygotic homeobox factor DUX4 and enriched at its targets during early development (Resnick et al. 2019). H3.Y2 also accumulated in osteosarcoma cells upon nutrient starvation and overgrowth stress, as well as in a subset of hippocampal neurons (Wiedemann et al. 2010) (**Figure 1**). The N-terminal lysines, K27 and K36, and the chaperone motif in the histone fold domain are conserved between H3.3 and H3.Y2, yet differences at other amino acid positions account for their exclusive recognition by HIRA but not by DAXX (Kujirai et al. 2016). This leads to deposition of H3.Y2 together with H3.3 at active genes in a heterotypic nucleosome (Zink et al. 2017). Whether H3.Y1 and H3.Y2 are expressed in DUX4-reactivating cancers and could be involved in their DUX4-dependent immune escape (Chew et al. 2019) would be important topics to explore.

The H3.5 variant, encoded by the intron-less *H3F3C* gene (Schenk et al. 2011, Shiraishi et al. 2018), is highly and specifically expressed during spermatogenesis (Schenk et al. 2011, Urahama et al. 2016). H3.5 differs from H3.3 at five amino acids, including I89V in the motif orienting the deposition mode (**Figure 2**). This feature makes it similar to both H3.1 and H3.3 variants. This chimeric recognition motif of H3.5 raises questions about its escort, as it might confer versatile interactions with different H3 chaperone complexes. As H3.5 is situated in the fragile 12p11 region, its copy number is increased in testicular germ cell tumors with frequent alteration of isochromosome 12p (iChr12p) (Törzsök et al. 2023). Whether this results in higher expression of H3.5 and particular chromatin features in those tumors needs further consideration. Rare *H3F3C* mutations also occur in acute myeloid leukemia (Boileau et al. 2019), melanoma, endometrial cancer, and non-small-cell lung cancer (Nacev et al. 2019), calling for further characterization of its regulation, expression, and escort by chaperones in different settings.

Importantly, many other testis-specific genes are overexpressed in cancers. Although not formally considered a histone variant, the reproductive tissue-specific enhancer of zeste homolog inhibitory protein (EZHIP)/CXorf67 contains a sequence homologous to H3K27M (Pajtler et al. 2018) and is worth considering here. This finding expands the possibilities for reshuffling oncochromatin landscapes beyond the sole mutations in histone genes (Jenseit et al. 2022). It also calls for a broader consideration of oncohistone mimetics in the context of disease.

## 3. HISTONE H3 ALTERATIONS IN CANCERS

### 3.1. H3 Oncohistones

The H3 hotspot substitutions in the histone H3 N-terminal tail occur mostly in young patients (Schwartzentruber et al. 2012, Wu et al. 2012, Behjati et al. 2013). In thalamic and hemispheric pHGG and in midline DIPG, these mutations hit mainly the nonreplicative variant H3.3 (*H3F3A* gene specific) and its chaperone ATRX and, more rarely, the replicative variants H3.1 or H3.2 (**Figure 3**). Further studies quickly segregated H3.1/H3.2- and H3.3-mutated malignant glioma on the basis of different cells of origin, brain locations, molecular pathways, and co-occurring alterations (Mackay et al. 2017, Jessa et al. 2022). The respective chromatin landscapes differed



significantly from each other when the mutation affected H3.1 or H3.3: H3.1K27M was distributed genome wide, whereas H3.3K27M accumulated mainly at sites of active histone turnover (Sarthy et al. 2020). These differences are consistent with the distinct properties of H3 histone variants in their expression and incorporation modes. The spatial and temporal resolutions of mutation burden evolution in DIPG tumors implied an early appearance and a driver role for H3.1K27M and H3.3K27M oncohistones in both tumor initiation and progression (Nikbakht et al. 2016). In contrast, H3.3G34R oncohistone proved to be important for initiating characteristic molecular alterations (Udugama et al. 2021), but both H3.3G34R/V mutations turned dispensable for tumor maintenance in pHGG (C.C.L. Chen et al. 2020). In the latter, recurrent platelet-derived growth factor receptor alpha (PDGFRA) alterations were under strong positive selection during tumor evolution (C.C.L. Chen et al. 2020). More recently, a subset of posterior fossa ependymoma A (PFA), which is another brain tumor with midline or cerebellar location, found in young children, also exhibited the characteristic genome-wide depletion of the H3K27me3 mark. Yet less than 5% of these tumors harbored a H3K27M mutation. The vast majority of PFA upregulated the germ cell-specific factor EZHIP, which acts as a natural oncohistone mimetic through a conserved H3-like peptide, corresponding to the K27M region (Pajtler et al. 2018, Jain et al. 2019, Jenseit et al. 2022). In long bone epiphyseal cancers, all mutations concern the H3.3 variant only, but the outcomes are strikingly different depending on the gene mutated and amino acid affected. The *H3F3A* mutations resulting in G34W/L substitutions represent a typical hallmark of giant cell tumor of bone (GCTB), while the *H3F3B* missense mutation K36M is instead found in chondroblastoma.

These peculiar associations raise questions about the developmental origins of oncohistone mimetics and variant-specific roles in pediatric pathophysiology. The identification of histone H3 somatic mutations in several other types of cancers followed shortly after (Nacev et al. 2019). In a small fraction of adult cancers, namely acute myeloid leukemia, H3K27M substitutions associated with *RUNX1* mutations, although not exclusively (Boileau et al. 2019). They involved mutations of other H3 genes and variants, such as *H3F3C* encoding H3.5, and other protein parts, notably the histone fold globular domain (Nacev et al. 2019). Histone H3 fold missense alterations reshuffle the chromatin landscape via a distinct mechanism and could potentially loosen transcriptional regulation of developmental programs by affecting nucleosome stability in a manner reminiscent of switch/sucrose non-fermentable (SWI/SNF) (Arimura et al. 2018, Nacev et al. 2019). While the concept of oncohistones started with histone H3, the focus chosen for this review, similar mechanisms driven by mutations in other histone genes involving, for example, H2A and H2A.Z could also affect chromatin and the epigenome (Arimura et al. 2018).

In addition to somatic mutations, germline mutations were recently identified in genes encoding H3.3 in patients with neurodevelopmental disorders (NDDs) without reported cancers (Bryant et al. 2020). Germline mutations were distributed broadly, with few overlaps with cancer hotspot mutated residues (Table 1). The R83C substitution alters a residue that directly contacts the DNA, potentially destabilizing the H3.3–DNA interactions (Bryant et al. 2020). R83 is also substituted in some cancers, but on the H3.1 variant (H3.1R83A/C), and is associated with a decreased rate of nucleosome sliding (Bagert et al. 2021). Given the expanding rate of discovery of H3 gene mutations in cancers and in other pathologies (Nacev et al. 2019, Bryant et al. 2020), one may expect some currently apparent differences between somatic and germline mutations, or between H3 variants, to fade over time. Importantly, histone H3 syndromes could inform on the developmental consequences of H3 mutations in cancers, as similar chromatin rewiring and stalled development circuits might be hijacked by cancer programs. Thus, the histone mutations offer a paradigm to link development and cancer.

**Table 1 Histone H3 residues altered in both cancers and NDDs**

H3 residue	Somatic missense mutation (in cancer)	Germline mutation (in NDDs)
R8	Some <i>H3F3C</i> H3.5R8H (AML)	<i>H3F3A</i> H3.3R8G/S <i>H3F3B</i> H3.3R8C
G34	Hotspot <i>H3F3A</i> H3.3G34R/V* (pHGG) Hotspot <i>H3F3A</i> H3.3G34L/W (GCTB) Some <i>H3F3A</i> or <i>H3F3B</i> H3.3G34L/W (OS)	<i>H3F3B</i> H3.3G34V*
K36	Hotspot <i>H3F3B</i> H3.3K36M (CB, HNSCC)	<i>H3F3A</i> H3.3K36E
R83	Some <i>HIST1</i> H3.1R83A/C* (e.g., EC, CC, HNSCC)	<i>H3F3A</i> H3.3R83C*

Common substitutions, found in both cancers and NDDs, are indicated with an asterisk. Strikingly, whereas H3.3G34V is recurrently mutated on the *H3F3A* gene in glioma, it is found on the *H3F3B* gene in NDD. Rare H3.1R83C substitutions, all hitting the *HIST1* locus, are found in several cancers, whereas the corresponding residue on the H3.3 variant is found in NDDs.

Abbreviations: AML, acute myeloid leukemia; CB, chondroblastoma; CC, colorectal cancer; EC, endometrial cancer; GCTB, giant cell tumor of bone; HNSCC, head and neck squamous cell carcinoma; NDD, neurodevelopmental disorder; OS, osteosarcoma; pHGG, pediatric high-grade glioma.

### 3.2. Other H3 Alterations

Loss of heterozygosity or methylation of the histone locus *HIST1* on chromosome 6 occurs in nearly 20% of a subtype of acute lymphoblastic leukemia (Holmfeldt et al. 2013, Tiberi et al. 2015). Intriguingly, 89% of testicular tumors present frequent iChr12p alteration (Fichtner et al. 2021), including focal *H3F3C*/H3.5 amplification (Törzsök et al. 2023). *EZH1P* is situated in the Xp11.2 region, which is frequently coamplified with chromosome 1p11 containing the *KDM4A* gene, encoding a H3K9/K36 demethylase (Black et al. 2013). Whether these focal amplifications comprise *H3F3C* or *EZH1P* genes deserves further investigation, as changes in their expression in tumors may alter chromatin organization.

Genetic alterations of the histone H3 variant CenH3/CENP-A are rare in cancers. However, its overexpression is a hallmark of almost half of human cancers, among which many solid tumors, such as breast, liver, colon, ovarian, and gastric, also exhibit *TP53* mutations (reviewed in Renaud-Pageot et al. 2022). In a p53-proficient context, CENP-A overexpression led to senescence and sensitivity to ionizing radiation, whereas in a p53-defective context, it promoted epithelial-to-mesenchymal transition (EMT) and the acquisition of chemoradiation resistance (Jeffery et al. 2021). In normal tissues and benign lesions, CENP-A localizes at the nuclear periphery in 9–18 foci homogeneous in size. These foci correspond to the clustering of several centromeres from two to five distinct chromosomes. However, in invasive neoplastic lesions, localization at the nuclear periphery is disrupted and the size and number of the CENP-A foci become highly heterogeneous (Verrelle et al. 2021). Thus, CENP-A and centromere subnuclear localization proved to be a reliable biomarker of cancer progression in several epithelial cancers, with a predictive value for radioresistance in patients with head and neck squamous cell carcinoma (Verrelle et al. 2021). Discrete dormant cellular populations such as senescent cells and quiescent cancer stem cells also contribute to tumor heterogeneity and represent a major obstacle for efficient patient treatment because they resist proliferation-targeting anticancer strategies. Moreover, quiescent subpopulations fuel a reservoir of cancer cells for relapse (Morales-Valencia & David 2022). Understanding how CENP-A levels are regulated and how quiescent cells maintain their centromeres in the absence of cell division, or upon cell cycle reentry, could provide insights into targeting them more efficiently. Although in G0 phase, quiescent cells require active CENP-A deposition and turnover at their centromeres, which were shown to be necessary for proper chromosome segregation upon reentry into the cell cycle (Swartz et al. 2019).

### 3.3. H3 Alterations and Cancer Cell of Origin

Best characterized in pHGG, histone gene mutations occur in a somatic manner without a hereditary component and likely during fetal life. They involve distinct transcriptional programs that correlate with their subsequent tumor location (Bjerke et al. 2013), competence to respond to regional cues (Bressan et al. 2021), and cell-type-specific differences in inducing chromatin changes (Haag et al. 2021), as detailed below. H3K27M mutation led to a similar global reduction of H3K27me3 in both brainstem and forebrain human fetal neural stem cells but initiated a proliferative phenotype only in the former, indicating that oncogenic cues co-opt a cell-specific window of opportunity for transformation (Bressan et al. 2021). Comparison of tumor transcriptomes, epigenomes, and 3D chromatin architecture in pontine and thalamic glioma subtypes bearing a K27M mutation on genes encoding either H3.1 or H3.3 showed that those tumor subtypes, despite their distinct cells of origin, faithfully preserved their memory of regional identity (Jessa et al. 2022). In line with their thalamic or pontine location, H3.3-mutated tumors showed a specific thalamic OTX1/2-positive or a mixed glial oligodendrocyte progenitor cell (OPC)/astrocyte signature, respectively (Jessa et al. 2022). H3.1-mutated tumors were characterized by a mixed early ventral OPC/ependymal signature, and posterior fossa ependymoma with the K27M-mimetic EZHIP expression showed an ependymal and, to some extent, glial progenitor signature (Jessa et al. 2022). By contrast, H3.3G34R/V-mutated hemispheric glioma, which most likely originates from interneuron progenitors, exhibited an impaired differentiation, with a striking reprogramming toward an astrocytic gliomagenic signature (C.C.L. Chen et al. 2020). This cell state transition and major chromatin rewiring result from the overexpression of, along with activating mutations in, the PDGFRA oncogene (C.C.L. Chen et al. 2020). Thus, H3K27M or EZHIP oncogenes preserve the transcriptional signatures of their cells of origin, whereas the H3.3G34R oncohistone reinforces forebrain progenitor circuits and shifts the cell of origin's transcriptional program from a neuronal toward a glial progenitor cell identity.

Finally, epiphyseal tumors harboring H3.3 missense mutations comprise chondroblastomas, which are benign tumors of immature cartilage tissues, and the locally aggressive highly recurrent GCTB. As only the stromal component contained H3.3 alterations, it most likely represents the neoplastic driving cellular population in both chondroblastoma and GCTB (Behjati et al. 2013, Venneker et al. 2020).

It will be important to explore whether oncohistone mutants in brain and bone developmental cancers are linked to the state of the cell and tissue of origin and their responsiveness to bone morphogens (Jessa et al. 2022)—a class of the transforming growth factor  $\beta$  (TGF $\beta$ ) family factors involved in both bone and brain development.

## 4. GENETICALLY ENGINEERED IN VIVO MODELS OF H3 ONCOHISTONE EFFECTS

Biopsies of tumors harboring oncohistone mutants offer access to advanced heterogeneous cancers, but information concerning the earlier stages of tumor development remains limited. In vivo disease models provide a powerful system with higher cellular and tissue complexity, necessary to model the etiology and development of brain tumors, and offer means for intervention to evaluate drug efficacy and toxicity in preclinical trials (Simeonova & Huillard 2014).

### 4.1. Mouse Models of Glioma

Constitutive H3.3K27M expression from the endogenous *H3f3a* locus resulted in early embryo lethality (Pathania et al. 2017), and its neonatal expression alone failed to initiate tumors (Larson et al. 2019). These observations indicate that modeling H3.3 oncohistone tumorigenesis

necessitates both a tumor-sensitized genetic background, through a combination with other frequently co-occurring alterations, and relevant regional cues. Alternative introduction of H3.3K27M combined with *Trp53* (encoding mouse p53) loss by small hairpin RNA, by in utero electroporation of plasmid constructs in forebrain and hindbrain, led to tumor formation in both locations. Further combination with *ATRX* inactivation or *PDGFRA* expression in the forebrain fastened tumor onset and increased the diffuse phenotype in the latter context (Pathania et al. 2017). However, expression plasmids could abrogate the need for specific signaling and do not address the H3.3 endogenous regulation in specific cell types, which could account for the regional and tumor segregation of H3 alterations in patients. More recently, Larson et al. (2019) established a genetically engineered mouse model expressing a nestin-Cre-recombinase inducible H3.3K27M mutation from the endogenous *H3f3a* allele, preserving its regulation. This enabled the neonatal expression of H3.3K27M oncohistones in the nestin-positive neural progenitors. Further combination of H3.3K27M with loss of *Trp53* and expression of an activating PDGFR $\alpha$  mutant protein accelerated spontaneous formation of brainstem glioma in vivo (Larson et al. 2019). However, combining H3.3K27M expression and p53 inactivation, in the absence of mutant PDGFR $\alpha$  protein expression, instead resulted in medulloblastoma incidence. Given that different mutational combinations and developmental variables could result in distinct tumors, the choices of developmental window, cell type, and combination of genetic lesions are crucial parameters to take into consideration. Yet in the two studies above (Pathania et al. 2017, Larson et al. 2019), the resulting mouse tumors faithfully recapitulated the molecular signature and transcriptional profiles characteristic of human glioma, providing tools for future preclinical studies and drug development.

A recent study reported a H3.3G34R substitution in a mouse model of glioma, in which the H3.3 mutation was combined with a loss of *Trp53* and PDGFR $\alpha$  expression in an *ATRX*-proficient or *ATRX*-deficient background (Abdallah et al. 2023). This finding further highlights the role of *ATRX* in regulating neural cell identity and its cooperation with different oncohistone variants during gliomagenesis.

## 4.2. Nonmammalian Models

Nonmammalian systems that exhibit evolutionarily conserved molecular mechanisms represent a complementary approach to grasp the molecular and cellular consequences of H3 oncohistones in a developing organism. H3K27M expression led to aberrant cell divisions in several tissues from different organisms. In *Drosophila* eye primordia, H3.3K27M expression resulted in disorganized tissue formation, impaired differentiation, and overgrowth of phenotypes, reminiscent of tumors (Herz et al. 2014, Ahmad & Henikoff 2021, Chaouch et al. 2021). A H3.3K36M mutant led to comparable histological phenotypes (Chaouch et al. 2021). At the chromatin and molecular levels, these lysine-to-methionine substitutions resulted in perturbed transcription of eye developmental genes and increased piwi-interacting RNA, a redistribution of H3K36me2 marks away from pericentromeres, and reactivation of transposable elements (Chaouch et al. 2021).

Ubiquitous H3K27M expression in the nematode promoted ectopic replication of germ cells, endomitosis, and DNA damage without another overt somatic manifestation (Delaney et al. 2019). In the mouse model from Larson et al. (2019), H3.3K27M-expressing neural progenitor cells showed an increased proliferation in culture conditions; however, aspects of cell cycle, DNA damage, and replication, or effects in tissues other than brain, were not examined in vivo. Importantly, the aberrant replication phenotype of germ cells in the nematode further identified upregulation of the autophagy and oxidative stress regulator Jun NH2-terminal kinase as a promising target in H3K27M-mutated patient-derived pediatric glioma cells (Delaney et al. 2019). Thus,

nonmammalian systems can be relevant to exploring oncohistone-induced epigenetic changes and vulnerabilities.

Lowe et al. (2021) compared a panel of yeast mutants with different H3G34 mutants. Strikingly, the different H3G34 substitutions recurrently found in cancers, namely arginine, leucine, or tryptophan, led to differences in epigenome reprogramming of histone PTMs and genome maintenance, promoting resistance to irradiation or defects in DNA replication and repair (Lowe et al. 2021). The recent establishment of a humanized yeast library for high-throughput profiling of a panel of histone mutation phenotypes (Bagert et al. 2021) represents an important resource. It allows researchers to compare at a large scale oncohistone mutations, their effects on chromatin remodeling, and their cellular properties.

## **5. HISTONE CHAPERONES AND CHROMATIN REMODELERS: LINK TO HISTONE VARIANTS AND DEREGULATION IN CANCERS**

### **5.1. Epithelial-to-Mesenchymal Transition**

Previous studies have implicated CAF-1, the chaperone for H3.1 and H3.2 histone variants, as a guardian of somatic cell identity, at least in part by regulating chromatin accessibility (Cheloufi et al. 2015). EMT promotes cell motility and differentiation as well as resistance to apoptotic stimuli during normal early development and is frequently co-opted by cancer cells to promote metastatic formation and dissemination (Thiery 2002, Pastushenko & Blanpain 2019). Recently, low levels of CAF-1 and a switch in histone H3 variant dynamics in response to activated ERK signaling stood out as drivers of cell identity transition toward invasiveness and metastasis and suggested histone chaperones as therapeutic targets in invasive carcinoma (Gomes et al. 2019). In this context, H3.3 accumulation and chromatin incorporation by its chaperone complex HIRA at EMT-specific genes favored their transcription, further fueling the metastatic transition (Gomes et al. 2019). *H3F3A* somatic overexpression in lung cancer also led to cell invasion, relapse, and poor prognosis (Park et al. 2016). This finding indicates that proper H3 histone dosage and chromatin organization are key to determining transcriptional outcome and cancer cell fate plasticity (Simeonova & Almouzni 2019).

### **5.2. Proliferation**

In contrast to the low expression of CAF-1 in metastatic cancers, high levels of CAF-1 correlated with the proliferative status in breast cancer samples (Polo et al. 2004). Furthermore, another H3 chaperone, ASF1, and specifically the ASF1B isoform, the expression of which is restricted to proliferative tissues and preferentially interacts with CAF-1 (Abascal et al. 2013), also proved to be a relevant biomarker with progression prognostic value in breast (Corpet et al. 2011), cervical (Liu et al. 2020), gastric (Chen et al. 2022), and lung (Wang et al. 2022) cancers and in a pan-cancer analysis assessing 22 types of cancers (Hu et al. 2021).

### **5.3. Chromatin Remodeling**

The ATRX/DAXX chaperone complex is defective in virtually all H3.3G34R/V tumors (Schwartzentruber et al. 2012). It is involved in the management of the H3.3 variant and its incorporation at the telomeric and pericentromeric heterochromatin, possibly via the recognition of the mark H3K9me3 (Iwase et al. 2011, Carraro et al. 2023). ATRX belongs to the SWI/SNF ATP-dependent family of chromatin remodelers, yet it displaces nucleosomes differently when compared with other SWI/SNF components (Xue et al. 2003).

Nucleosome remodeling by SWI/SNF complexes is fine-tuned by their subunit composition and sensitivity to PTM landscape signatures, including H3K4me and H3K27ac (Mashtalir et al.

2021). This is particularly intriguing given the high levels of H3K27ac characteristic of H3K27M-harboring tumors (Lewis et al. 2013), as H3K27ac could interfere with proper functioning of specific SWI/SNF subunits in those tumors (Mashtalir et al. 2021).

The interaction of H3K27M oncohistones directly with the H3K4 methyl mark writers MLL1 and MLL2 could catalyze H3K4me3 enrichment at long terminal repeats (LTRs), resulting in their derepression (Furth et al. 2022). Given the repressive role of p53 in LTR regulation (Wylie et al. 2016, Tiwari et al. 2020), the frequent *TP53* mutations in glioma with H3K27M may cooperate with H3 oncohistones to impair the distribution of H3K4 methyl marks and transcription. In this context, it would be worth exploring in glioma the previously reported interaction of mutant p53 with MLL4, which resulted in H3K4me1 redistribution and aberrant enhancer activity in other situations (Rahnamoun et al. 2018).

In contrast, H3 mutations affecting the histone fold domain, such as H3E105K, as well as mutations in histones H2A.Z and H2B, were characterized as SWI-independent (Sin-), as they induced nucleosome instability similar to SWI/SNF nucleosome remodeling (Arimura et al. 2018, Nacev et al. 2019).

#### 5.4. Alternative Lengthening of Telomeres

Most cancers escape replicative senescence by reactivating the expression of telomerase, the ribonucleoprotein complex involved in the maintenance of chromosome ends. However, 10–15% of cancers use another immortalization strategy: alternative lengthening of telomeres (ALT) (Hoang & O'Sullivan 2020). The depletion of all ASF1 isoforms induced hallmarks of ALT in both primary and human cancer cells (O'Sullivan et al. 2014). Mechanistic studies further identified a role for ASF1, in complex with the telomere-binding protein RIF1, in protecting DNA broken ends and stimulating their repair by nonhomologous end joining (Feng et al. 2022, Tang et al. 2022). ALT is also a hallmark of pHGG bearing H3.3G34R/V oncohistones. These tumors exhibit major global chromatin demethylation mainly in nonpromoter regions, including telomeres and repetitive elements, and a high-frequency telomere dysfunction (Sturm et al. 2012, Udugama et al. 2021). Compromised deposition of H3.3 by ATRX/DAXX results in chromatin accessibility of those regions and in chronic DNA damage signaling (Juhász et al. 2018). Alterations of *ATRX* are not specific to H3 mutant pHGG and are at the root of several glioma molecular classifications, including a subset of adult low-grade infiltrative glioma. Adrenal and extra-adrenal gland tumors, such as pheochromocytomas and paragangliomas, are also characterized by *ATRX* alterations, telomere instability, and ALT mechanisms (Fishbein et al. 2015). Thus, the interconnection between H3 oncohistones and chaperones and ALT pathways deserves further attention.

## 6. TARGETING H3 ONCOHISTONES AND EPIGENETIC LESIONS IN TUMORS

Bone cancers do not exhibit malignant behavior, although GCTB is locally aggressive. The current therapies for bone tumors include surgery, when the tumor is accessible, and curettage combined with local bone grafting. pHGGs, on the contrary, are malignant deadly tumors of the brain. The hemispheric pHGG is treated with surgery and a combination of radiotherapy and chemotherapy. The midline location of DIPG often prohibits surgical intervention and is treated with radiotherapy. Several efforts (see below) have focused on identifying new effective strategies, as the median survival of patients who receive current treatments remains less than 1 year (Jones et al. 2017).

In a chemical screen of patient-derived DIPG cell lines, the histone deacetylase (HDAC) inhibitor panobinostat stood out among the most effective agents, reducing cell viability and tumor

growth following orthotopic engraftment in mice (Grasso et al. 2015). However, HDACs have low substrate specificity extending beyond histones, leading to their pleiotropic effects.

The discovery of recurrent somatic missense mutations of H3 oncohistones, beyond being an important diagnostic tool, opens opportunities to exploit cancer-specific epigenetic lesions for targeted therapies with a minor effect on nontumoral tissues. The recent demonstration that H3K27M is essential for gliomagenic properties in cell lines and in patient-derived xenografts positions H3K27M oncohistone as both a cancer driver and its Achilles' heel (Harutyunyan et al. 2019) (**Figure 4**). H3K27M-bearing tumors exhibit a global loss of H3K27me<sub>3</sub>. A strategy to counteract their oncogenic effect would be to inhibit the H3K27me<sub>3</sub> histone demethylases JMJD3 and UTX, as demonstrated for the small-molecule inhibitor JSKJ4 (Hashizume et al. 2014). Despite the global loss of H3K27me<sub>3</sub>, some strong Polycomb repressive complex 2 (PRC2) targets, such as the *CDKN2A* locus encoding the tumor suppressors p16 and p14, retain H3K27me<sub>3</sub>, leading to their epigenetic silencing. In line with these findings, *CDKN2A*-reactivating strategies, using allosteric PRC2 inhibitors such as EED226 (Qi et al. 2017) and MAK683 (Chu et al. 2022), inhibited cell growth and induced either cellular senescence or differentiation, depending on the tumor context (Mohammad et al. 2017). H3K27M-related chromatin changes also included an aberrant increase in H3K36me<sub>2/3</sub> marks that accompanied H3K27me<sub>3</sub> loss (Yu et al. 2021). Thus, targeting H3K36me<sub>2/3</sub> writers and readers represents another dependency to explore for potential pharmacological development (Yu et al. 2021) (**Figure 4**).

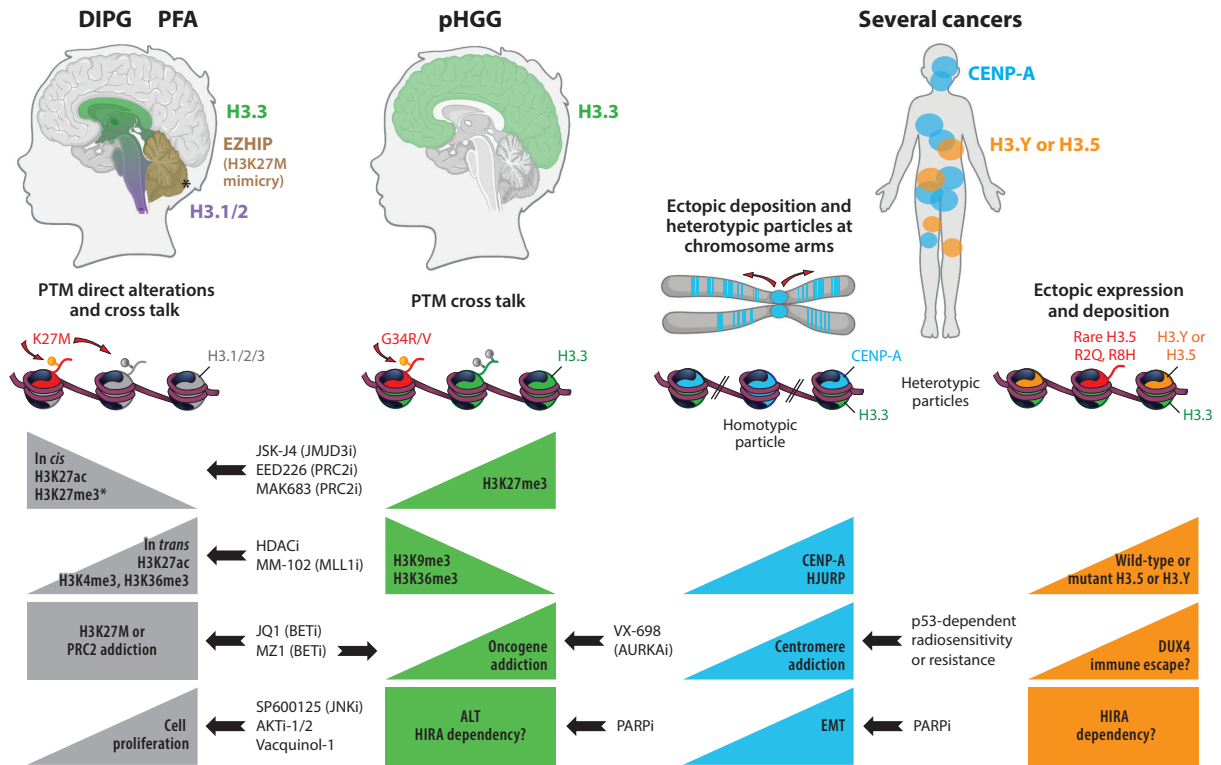
Heterotypic nucleosomes containing both H3 mutant H3K27M and wild-type H3K27ac interacted with the bromodomain and extraterminal domain (BET) transcriptional activators BRD4 and BRD7 and excluded PRC2 from transcriptionally active chromatin regions. The BET bromodomain inhibitor JQ1 efficiently targeted H3K27M-positive glioma cells, further promoting their neuronal differentiation (Piunti et al. 2017). Novel BET inhibitors of BRD4, such as MZ1, were also effective at suppressing the MYC-N oncogene, leading to inhibition of MAPK signaling (Zhang et al. 2022). MYC-N is highly expressed in H3.3G34V pediatric glioma cells and its destabilization reduced tumor cell viability (Bjerke et al. 2013). Thus, the use of BET inhibitors represents a promising strategy for targeting glioma harboring different H3 substitutions on either K27 or G34 residues (**Figure 4**).

In vivo models of H3K27M tumors are also valuable preclinical tools. A screen of small-molecule kinase inhibitors identified vacquinol-1 and the AKT inhibitor Akti-1/2 as promising therapeutic strategies (Pathania et al. 2017). Moreover, H3K27M expression in the nematode, which led to ectopic DNA replication and chromatin dysfunction, identified the JNK inhibitor SP600125 as an interesting selective strategy to target H3K27M-positive cells (Delaney et al. 2019) (**Figure 4**).

Finally, on another frontline, EZHIP is highly expressed in a subset of ependymoma; however, its restricted expression pattern in normal tissues offers opportunities to specifically target cancer cells, with minor effects on normal tissues. A similar strategy could be applied to the tissue-specific and stress-inducible H3.Y2 and its transactivator DUX4 (**Figure 4**). Strikingly, DUX4 reactivation promoted cancer immune escape (Chew et al. 2019). A more thorough understanding of the functioning, regulation, and consequences of EZHIP, H3.Y, and DUX4 will increase the range of therapeutic opportunities.

## 7. CONCLUDING REMARKS AND PERSPECTIVES

Cell identity, chromatin states, and genome integrity are intimately linked. During cellular transformation, undifferentiating transcription circuits are reinforced at the expense of normal differentiation trajectories. Thus, reshaping oncochromatin challenges genome function and integrity. The acquisition of oncogenic properties in tumor cells is also accompanied by



**Figure 4**

Epigenetic deregulations and associated vulnerabilities in cancers with altered H3 histone variants. Tumor locations associated with alterations of specific H3 variants are indicated by color: gray for H3.1-, H3.2-, or H3.3-containing nucleosomes; green for H3.3-containing nucleosomes; blue for CENP-A-containing nucleosomes; and orange for H3.Y2- or H3.5-containing nucleosomes. Mutant H3 histone variants are in red. PTMs are depicted as balls. Epigenetic alterations and molecular and cellular phenotypes are summarized for each tumor type. H3K27M substitutions directly interfere and prevent key H3K27 PTMs, such as acetylation and methylation on the mutant histone H3 tail. The H3K27M-mimetic EZHIP, without being incorporated into chromatin, sequesters PRC2 and results in a global decrease of H3K27me3 (indicated with an *asterisk*), similar to the effect of the H3K27M oncohistones. Moreover, H3K27M and H3.3G34R/V oncohistones interfere with different PTMs on nearby residues, both on H3 mutant tails and on H3 wild-type tails, leading to local and global chromatin changes. These changes can represent vulnerabilities to explore for effective targeting of cancer cells. As PFA share common chromatin deregulations with DIPG (PRC2 inhibition and H3K27me3 decrease genome wide), their treatment could potentially benefit from therapeutics developed to target the H3K27M-associated alterations. BET inhibitors represent a common oncohistone-targeting strategy between H3K27M (targeting of PRC2) and H3.3G34R/V-harboring cancers (targeting of MYC-N). Mutations in genes encoding CENP-A, H3.Y1/2, and H3.5 are rare in cancers; however, the ectopic expression and deposition of these histone variants in heterotypic nucleosome particles (composed of two different H3 variants) result in chromatin remodeling. Overexpression of centromere factors confers sensitivity to ionizing radiation in p53-positive cancers and is associated with radioresistance in p53-negative cancers. Therapeutic targeting of H3 variants in cancers with H3.5 or H3.Y1/2 deregulation needs further development. Chaperone specificity (HIRA dependency) or transcriptional regulation (DUX4 expression) would be interesting to explore in this context. Abbreviations: ALT, alternative lengthening of telomeres; AURKA, Aurora kinase A; BET, bromodomain and extraterminal domain; DIPG, diffuse intrinsic pontine glioma; EMT, epithelial-to-mesenchymal transition; EZHIP, enhancer of zeste homolog inhibitory protein; HDAC, histone deacetylase; HIRA, histone regulator A; HJURP, Holliday junction recognition protein; i, inhibitor; JNK, Jun NH2-terminal kinase; MLL1, myeloid/lymphoid or mixed-lineage leukemia 1; PARP, poly(ADP-ribose) polymerase; PFA, posterior fossa ependymoma A; pHGG, pediatric high-grade glioma; PRC2, Polycomb repressive complex 2; PTM, posttranslational modification. Figure adapted from images created with BioRender.com.



vulnerabilities representing cancer's Achilles' heel: addiction to oncogenes (Weinstein 2002), to epigenetic factors (Hubert & Lathia 2021), and to aneuploidy (Girish et al. 2023). Importantly, cancers linked to oncohistones such as H3K27M maintained defective chromatin (Harutyunyan et al. 2019), leading to aberrant redistribution of H3K27me3 (Mohammad et al. 2017) and H3K36me2/3 (Yu et al. 2021) marks and associated vulnerability. In H3.3G34R/V-mutated glioma, loss of ATRX/DAXX results in ALT and a strict dependency on HIRA for onco-H3.3 deposition (Schwartzentruber et al. 2012), and the PDGFR $\alpha$  oncogene co-option rewires their neuronal identity (C.C.L. Chen et al. 2020). Finally, p53-negative cancers are addicted to high levels of centromeric proteins such as CENP-A and HJURP and exhibit radioresistance (Filipescu et al. 2017, Renaud-Pageot et al. 2022) (**Figure 4**). These various changes also contribute to tumor heterogeneity, stem or metastatic properties, and reactivity to the tumor environment. As these aspects are major obstacles for effective cancer therapies, the use of differentiation strategies combined with targeting of specific epigenetic lesions offers promising avenues. Nevertheless, a key challenge for earlier interventions or interception is to characterize defects early on and to detect risk of recurrence. Cellular interception medicine is surely an area worthy of consideration (Rajewsky et al. 2020).

In addition, drug testing in in vivo systems and the development of devices that facilitate blood–brain barrier penetration will be crucial for effective drug delivery (Carpentier et al. 2016, Rahman et al. 2023). Furthermore, the establishment of methods that detect defects in blood will be crucial for noninvasive follow-up in patients. Collaborations between patients, physicians, innovative companies, and health care systems are needed for the development of next-generation personalized medicine (Subbiah 2023) to improve patients' lives.

## DISCLOSURE STATEMENT

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