Histone H3G34 Mutation in Brain and Bone Tumors

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Abstract

H3G34 mutations occur in both pediatric non-brainstem high-grade gliomas (G34R/V) and giant cell tumors of bone (G34W/L). Glioblastoma patients with G34R/V mutation have a generally adverse prognosis, whereas giant cell tumors of bone are rarely metastatic benign tumors. G34 mutations possibly disrupt the epigenome by altering H3K36 modifications, which may involve attenuating the function of SETD2 at methyltransferase. H3K36 methylation change may further lead to genomic instability, dysregulated gene expression pattern, and more mutations. In this chapter, we summarize the pathological features of each mutation type in its respective cancer, as well as the potential mechanism of their disruption on the epigenome and genomic instability. Understanding each mutation type would provide a thorough background for a thorough understanding of the cancers and would bring new insights for future investigations and the development of new precise therapies.

Keywords

Glioblastoma · Giant cell tumors of bone · Epigenome · Genomic instability · Oncohistones

5.1 Oncohistone H3G34R/V Mutation in Brain Tumors

H3G34 mutations mostly arise in brain and bone tumors. Depending on the tumor type, the amino acid substitutions are different. For example, *H3F3A* G34R/V mutations often occur in pediatric non-brainstem high-grade gliomas (HGGs), whereas *H3F3A* G34W/L mutations are usually observed in patients with giant cell tumors of bone (GCTB) [1–3].

H3G34R/V mutant tumors are typically located in the cerebral cortex in young adults with a median age of 18 years old, older than for H3K27M mutant tumors [4–7]. Almost all G34R/V mutations happened in *H3F3A*, and they almost always overlap with mutant TP53/ATRX [8]. However, one group in Japan demonstrated that G34R-mutant tumors might also occur in the basal ganglia and deep-seated region, away from the thalamus [9]. Meanwhile, they suggested that G34R/V mutations played a role in the pathogenesis of astroblastoma [9].

H3F3A G34-mutant HGGs arise as primary malignancies like most pediatric HGGs. There is no evidence for lower-grade precursors

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[10]. H3G34R mutant cases are characterized by poorly differentiated/PNET (primitive neuroectodermal tumors) -like tumor cells with very high pleomorphism and high cell density [11]. MRI features of H3G34 mutant diffuse gliomas may be very heterogeneous, where some cases may not even fulfill the imaging criteria of HGGs [12].

Glioblastoma (GBM) patients with G34R/V mutation have a generally adverse prognosis, with reported median progression-free survival (PFS) of 8–9 months and overall survival (OS) of 12–22 months [13, 14], still better than the PFS and OS for other GBM molecular subtypes (not including IDH1-mutant tumors) [15–17]. This may be explained by the frequent methylation in *MGMT* promoter, which relates to boosted temozolomide responsiveness [14].

It is not clear why G34V/R mutation preferably occurred on *H3F3A* over *H3F3B* and why R is a much more frequent mutant than V, since the G34 codon is the same in both genes. Meanwhile, single point mutations can cause both R and V substitutions [18].

5.2 Oncohistone H3G34W/L Mutation in Bone Tumors

H3F3A mutations were also identified in more than 90% of GCTB, leading to G34W/L substitutions [2, 3]. GCTB are young adult benign tumors that are locally aggressive and may cause extensive bone destruction [19]. In most of the cases, H3G34W/L mutations were only found in stromal cells but not in osteoclasts or their precursors [2].

More recent studies have identified G34W mutations in a cancer syndrome (including pheochromocytomas, paragangliomas, and GCTB) [20] where G34W mutation is thought to occur post-zygotically during development rather than as a somatic mutation, since G34W mutation was detected in distinct tumor types within the same individual, whereas their germline *H3F3A* was wild type [18]. A study based on GCTB-derived primary cell lines as well as isogenic knock-in *H3F3A* G34W and WT cell lines has indicated that the H3.3 G34W mutation promotes cell proliferation [21].

H3.3 G34W mutation occurs much more frequently may be because it requires only one base substitution alone, whereas H3.1 G34W or H3.1/H3.3 G34L mutations require substitution of at least two nucleotides [22].

5.3 Impact of H3G34 Mutation on the Epigenome

The functional mechanisms of H3G34 mutations in DNA replication-coupled nucleosome assembly and/or gene transcription are less straightforward to interpret than the mechanisms proposed for H3K27M/K36M mutations. Although H3G34 cannot be posttranslationally modified, it sits only two residues from K36, a residue that undergoes methylation during transcriptional elongation, and four residues from P38, a residue that adopts conformational change to control K36 methylation [23]. Therefore, it is possible that H3G34 mutations may influence K36 accessibility to histone-modifying complexes, thus modifying H3K36 methylation or acetylation. Generally, H3G34 mutations locally block H3K36 methylation on the same and nearby nucleosomes but do not dominantly inhibit bulk H3K36me3 [1, 24–26].

It is possible that introducing a bulky residue (W) or a charged (R) in place of G34 may lead to changes in the accessibility or activity of K36 modifying enzymes or H3K36me3 readers [22]. Indeed, in vitro assay had revealed that G34 mutants were unable for H3K36 methylation mediated by SETD2 [27], suggesting that mechanisms of H3G34 and H3K36M mutations may be similar, where they regulate target gene expression by attenuating SETD2 function in transcriptional elongation (Fig. 5.1). Ectopic expression of Flag-H3.3G34W/L mutant also resulted in increased H3K27me3, reduced H3K27ac, and reduced H3K9me3 levels compared to WT H3.3 [26]. H3.3G34L cells showed a more remarkable H3K27me3 increase than that in H3.3G34W cells, whereas H3K27ac has a little bit change [26]. Consistent with H3K27me3 and

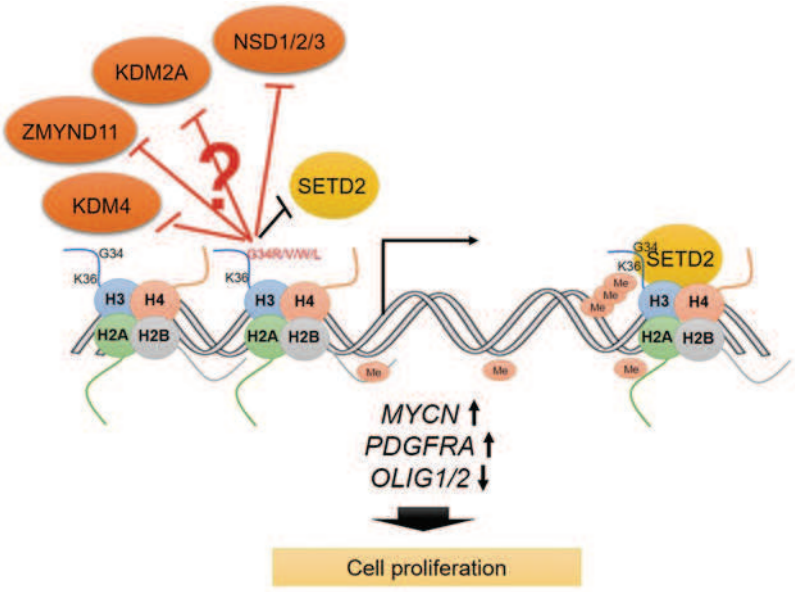

Fig. 5.1 A working model of the mechanism how G34 mutations might disrupt H3K36 methylation and gene expression

H3K36me3 changes, the binding of PRC1 (CBX8 and RING2) and PRC2 complex components (EZH2, SUZ12, and EED) is increased, while the binding of H3K36me3 reader (ZMYND11) to H3G34 mutants is reduced [26]. Interestingly, H3G34 mutations also inhibit histone binding to the H3.3 chaperone HIRA [26], whose complex member UBN1 is known to bind to the H3($A_{87}AIG_{90}$) segment that is far from the H3G34 region [28]. Subsequent studies also detected alterations in H3K27me3 and H3K9me3 in a subset of G34R mutant tumors [9, 26, 29]. G34 mutations generally do not affect methylation on H3K4 and H3K79 [26].

Fang et al. also showed that H3G34 mutations significantly reduced in vivo H3K36me2/me3 on the tail of mutant H3 and impacted in vitro function of SETD2, NSD1, and NSD2 [30]. In G34R mutant fission yeast model, Yadav et al. found that both H3K36me3 and H3K36ac levels decreased, whereas H3K36me2 amassed on the mutant tail [31]. It is difficult to separate out the effect of losing only K36me3 [18], since Set2 itself mediates all three levels of H3K36 methylation in fission yeast [32]. Mammalian studies showed consistent H3K36me3 reduction, suggesting that G34R disturbs SETD2-mediated K36 trimethylation. However, the accumulated H3K36me2 suggests that Set2 can still methylate the G34R-mutant H3 tail in fission yeast, consistent with results from ChIP studies showing that Set2 still interacts with G34R-mutant chromatin [31].

A sequencing study comparing pediatric HGGs to non-cancer samples identified *SETD2* mutations in 15% of pediatric HGGs [33], suggesting that disruption of H3K36 methylation is essential for HGG carcinogenesis. *SETD2* mutant HGGs localize to the cerebral hemispheres along with H3.3G34R/V mutant tumors and frequently contain *IDH1* mutations, suggesting that H3.3G34R/V and *SETD2/IDH1* mutations may function together to interrupt the modification of K36me3 in tumors [33].

Structural analyses revealed that the H3G34 residue was completely buried within a very narrow channel flanked by F1668 and Y1671 of SETD2, which could not hold a larger amino acid at position 34 [18, 30, 34, 35]. Although SETD2 and other SET domain methyltransferases share considerable sequence homology at this region [34, 35], F1668 and Y1671 seem to be essential for the size restriction of the G34 channel in SETD2 [18]. The reason why H3.3G34R/V mutant histones retained mono-methylation and some di-methylation of K36 may be explained by

the shorter side chains (Leu) at both positions in NSD1, 2, and 3, providing a larger channel in these methyltransferases [18, 25]. Surprisingly, while NSD1, 2, and 3 bind more strongly to the G34 mutant H3.3 than the wildtype nucleosomes, they are defective in K36 methylation on the G34 mutant histones [26]. Similar to SETD2, the H3G33-G34 motif occupies a narrow channel in KDM2A, a demethylase that has activity on K36me2/me1 but not on K36me3, G34 substitutions are thus predicted to attenuate KDM2A binding to H3 [36].

Since histones can be methylated on multiple arginine residues [37], Lowe et al. also tested if G34R mutant might provide a new methylation site on the histone tail [18]. They demonstrated in fission yeast that only 1% of the G34R peptide was methylated under enzyme excess conditions in vitro and the methylation on arginine residue of H3G34R mutant was not found in vivo [18]. Whether H3.3G34R is methylated in pediatric cortical HGGs is still obscure.

A recent study revealed that G34 mutants might also have dominant effects on H3 biology and sought to shed light on their mechanism [18]. The study conducted by Voon et al. found that H3G34R mutation in mouse embryonic stem cells triggered H3K36me3 increase at some genomic regions, though the overall H3K36me3 levels were unchanged [27]. They hypothesized that the K36me3 elevation was induced by KDM4 suppression and demonstrated in support that the transcriptional and K36me3 profiles of G34R-mutant cells were both similar to those of KDM4-deficient cells [27]. They also confirmed a correlation between the KDM4-binding sites and K36me3 accumulated regions in G34R cells [27]. Since KDM4 is a H3K9me3 demethylase, they noticed that H3K9me3 levels increased at KDM4-binding sites in G34R cells [27]. These results suggested a correlation between the loci altered by G34R mutation and the KDM4-binding sites.

One possible mechanism whereby this suppression in demethylase activity was that G34R mutation disrupted KDM4 interaction with H3.3 [18]. In consistence to this hypothesis, in vitro demethylation assays revealed that G34R mutant suppressed the KDM4A, B, and C demethylase activity toward H3K36me3 [27]. Structural studies indicated that the interaction between H3.3G34R mutant and KDM4 was weak, since KDM4 active site could not hold an amino acid bigger than glycine [38, 39]. This suggests that the reduction of KDM4 activity at its target genes may result in H3K36me3 enrichment and trans-effects on the chromatin landscape [18]. However, KDM4 immunopurification assay with cells expressing exogenous histones showed that KDM4s bound to G34R more tightly compared to wild-type H3.3 [27], indicating that KDM4 accumulation at G34R deposition sites might decrease H3K36me3 at these sites [18]. Therefore, KDM4 enrichment might be working in combination with the reduction of SETD2 activity at H3.3G34R loci, to guarantee a robust K36me3 loss [18].

ZMYND11, a tumor suppressor and a H3K36me3 reader, is another interesting protein that may be influenced by the changed local H3K36 methylation state [40, 41]. Although the function of ZMYND11 in pediatric HGGs or GCTB has not been demonstrated yet, it is found that H3G34V/R mutations disrupt the interaction between ZMYND11 and the H3.3K36me3 peptide [41]. ZMYND11 delocalization may contribute to the tumorigenesis of H3K36M and H3G34 mutant tumors by locally decreasing H3K36 methylation [22].

It is important to elucidate the detailed mechanisms of how H3G34 mutants affect the activities of SETD2 and other H3K36 methyltransferases, and how they may contribute to tumorigenesis. The reason why G34 instead of other K36 neighboring residues is targeted remains unknown. It would also be great curious to investigate whether ZMYND11 or SETD2 mutation arises in these tumor types and if their knockdown in the suitable cell types may recapitulate features of the diseases.

5.4 H3G34 Mutation and Genomic Instability

H3K36 methylation is important for genomic stability, since H3K36 modification may influence the choice of DNA damage repair pathway

between non-homologous end-joining (NHEJ) and homologous recombination (HR) [42]. H3K36 methylation status may also determine the timing of origin activity during DNA replication [43] and manipulate the mismatch repair pathway by recruiting hMutSα, a mismatch recognition complex, onto chromatin [44]. H3G34R/V mutant cells demonstrated slightly elevated mutation frequency, consistent with a reduced amount of chromatin-bound MSH6 due to decreased MSH6 affinity for binding the mutant H3 tail and K36me3 reduction in H3G34R/V cells [30]. Microsatellite instability is very high in pediatric HGGs [45]. A recent study has revealed that histone chaperon HIRA restores transcription after DNA damage repair by depositing the H3.3 histones into ultraviolet C (UVC) radiation-damaged regions [46]. Chicken bursal lymphoma DT40 cells harboring G34R/V mutant H3.3 or lacking histone H3.3 are sensitive to UV [47]. Interestingly, G34 and K36 mutations occur exclusively in H3.3 which plays a critical role in chromatin repair [48]. Pediatric HGG is distinguished by frequent somatic coding mutations, suggesting possible DNA damage repair deficiency [49]. Therefore, it may be beneficial to further investigate the influence of H3G34 mutations in cancer DNA damage pathways [22].

The pediatric HGG bearing H3G34V mutation has a great change of the genome-wide H3K36me3 pattern, leading to an altered transcriptional signature. ChIP-seq data identified 156 genes that were differentially enriched in RNA polymerase II and H3K36me3 in the KNS42 cell line (derived from a H3G34V mutant pediatric HGG patient) when compared to a *H3F3A* wild-type pediatric GBM cell line [1]. This gene set was enriched for regulators of forebrain and cortical development (*DLX6* and *FOXA1*) that were upregulated from embryonic and early fetal time points and downregulated by mid-late fetal development [50]. H3G34W expression provokes transcriptional dysregulation and altered splicing patterns, causing frequent exon inclusion, which may lead to freak transcript stability, open reading frame extension, and alternative start site usage to promote cell proliferation [21]. Aberrant H3K36me3 and RNA polymerase II enrichment may also result in the transcription of oncogenes or micro-RNAs with oncogenic functions as well as prevent the expression of tumor-suppressor, thus boosting the growth of the respective tumors [51]. Gene expression analyses also uncovered gene expression patterns that were different in samples with the H3K27M mutation versus samples with the H3G34R/V mutation, suggesting that each mutation favors a specific gene expression signature [51].

One of these dysregulated genes is the *MYCN* oncogene (Fig. 5.1). It has been shown that the forced overexpression of stabilized MYCN protein in neural stem cells of the developing mouse forebrain had an ability to produce GBMs [52]. Studies have also demonstrated the mechanism by which the initiating tumorigenic insult is delivered at the correct time and place [53] during neurogenesis. Therefore, targeting MYCN protein stabilization through inhibiting the responsible kinases in *H3F3A* G34-mutant pediatric GBM provides a potential novel approach to treat this subgroup of patients [1]. The potential link between H3.3 and MYCN is remarkable given the association of H3.3 with actively transcribed genes and the function of MYCN in the maintenance of global euchromatin in neural stem cells [54] and neuroblastoma [55].

All H3.3G34 mutant tumors are found to also bear the inactive mutations in the ATRX (alpha thalassemia/mental retardation syndrome X-linked protein)/DAXX (death domain-associated protein) chaperone complex, which facilitates H3.3 deposition into telomeric and pericentromeric regions. Demethylated DNA were found at chromosome ends in H3G34R/V mutant groups, indicating a connection between H3G34R/V mutations and ALT [1]. Therefore, H3G34R/V mutations may not simply alter H3K36me3 levels and activate potential cancer driver genes, but mutations in ATRX/DAXX or H3.3 may interrupt their proper interaction, resulting in abnormal H3.3 deposition near telomeric regions and causing ALT [56]. It is also found that partial H3.3 functional loss in the mouse led to genomic instability [57] and H3.3 is linked to functional complexes involved

in DNA double-strand break repair (DDR), HR [17, 58], and somatic hypermutation [59].

G34 mutant tumors tend to also have high frequency on *TP53* (88%) and *ATRX* (95%) alterations, as well as *PDGFRA* amplification (approximately 27%), 2q loss (67%), and 4q loss (70%) [6, 60]. Cytogenetic analysis comparing GBM subtypes revealed a highly frequent and specific 3q and 4q loss among H3G34 mutant tumors [14].

PDGFRA amplification was more frequently found in GBM cases than in cases with primitive neuroectodermal tumors (PNET) morphology (36% vs. 5%, respectively), whereas an opposite trend was found in *CCND2* amplification (5% vs. 27%) [14]. It is reasonable to speculate that the variability of these high copy number aberrations may contribute to the morphological heterogeneity in H3G34 mutant tumors [14]. But then, these aberrations are not general molecular events since they were identified in only less than 1/3 of the studied tumors [14].

H3.3G34 mutations are associated with global DNA hypomethylation, particularly prominent in sub-telomeric regions [61]. Promoter methylation differences led to differential expression of genes in a tumor origin-dependent manner, suggesting that both DNA methylation and gene expression may be a consequence of tumor origin altered by histone mutations [1, 61–63].

Despite the fact that DNA hypomethylation is typically related to increased gene expression, G34 mutations are responsible for downregulating the expression of differentiation genes [61]. Among these genes are a couple of members from the Olig transcription factor family [64]. For example, *OLIG1/2* hypermethylation leads to low OLIG1/2 expression levels in these tumors (Fig. 5.1). This pattern is very similar to that of embryonic stem cells, where epigenetic suppression of OLIG1/2 has been proposed as a barrier of neural lineage commitment [65]. Olig-2 is involved in early central nervous system development and linked to oligodendroglial differentiation. Moreover, it is commonly expressed in glioma cells and is considered as a glial differentiation marker [66]. In Schafer's study, using immunohistochemistry, they analyzed the nuclear Olig-2/FoxG1 expression in a large series of gliomas and found that K27M-mutant tumors carried a FoxG1low/Olig-2high and G34-mutant tumors showed a FoxG1high /Olig-2low profile, which was in accordance with previous data reporting in eight K27M-mutant tumors a Olig-2 +/FoxG1-immunoprofile and in six G34-mutant tumors an Olig-2-/FoxG1+ profile [61, 67].

Nevertheless, Olig-2 expression has recently been found in a low metastatic CNS-PNET subgroup (defined as "oligo-neural" group) as well [68]. Furthermore, the H3G34 mutant mRNA signature seems to demonstrate transcriptional patterns of early CNS developmental stages, suggesting a specific cellular origin and tumor initiation time [14]. Therefore, it may be indicated that the driving H3G34 mutation impacts poorly differentiated neuroepithelial embryonic cells or progenitors, which would develop either glial or neuronal differentiation patterns, respectively, hence explaining the morphological heterogeneity of H3G34 mutant HGG [14]. It is also possible that this particular expression pattern is an outcome from the reprogramming of the H3G34 mutation itself [14].

Chromatin is critical and fundamental for the control of DNA replication, DNA damage repair, gene transcription, and other aspects of genomic stability, including maintenance of telomere integrity and high-fidelity chromosome segregation during cell division [18]. H3.3G34 mutations disrupt H3K36 methylation, RNA polymerase II enrichment, and DNA methylation, leading to aberrant gene expression signature, DNA damage repair, and possibly disrupted chromatin stability and ALT. Further studies would be beneficial since detailed mechanisms of how H3.3G34 mutations dysregulate these biological processes are still unclear.

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