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Somatic Histone H3 Alterations in Paediatric Diffuse Intrinsic Pontine Gliomas and Non-Brainstem Glioblastomas

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Abstract

To identify somatic mutations in paediatric diffuse intrinsic pontine gliomas (DIPGs), we performed whole genome sequencing of 7 DIPGs and matched germline DNA, and targeted sequencing of an additional 43 DIPGs and 36 non-brainstem paediatric glioblastomas (non-BS-PGs). 78% of DIPGs and 22% of non-BS-PGs contained p.K27M mutation in *H3F3A*, encoding histone H3.3, or the related *HIST1H3B*, encoding histone H3.1. An additional 14% of non-BS-PGs had somatic p.G34R *H3F3A* mutations.

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Diffuse intrinsic pontine glioma (DIPG), an aggressive brainstem astrocytic tumour, arises almost exclusively in children, usually with histopathological diagnosis of glioblastoma, and has a long-term survival of less than 10% [1]. To understand the molecular pathogenesis of DIPG, we performed whole genome sequencing (WGS) on DIPGs and matched normal DNA from seven patients. Tumours from four patients had a recurrent somatic adenine to thymine transversion in *H3F3A* resulting in a substitution of methionine for lysine 27 (p.K27M) of histone H3.3 (Supplementary Figure 1). In the tumour from a fifth patient, an analogous A to T transversion encoding p.K27M mutation was identified in the closely related *HIST1H3B*, encoding an isoform of histone H3.1.

To determine the frequency of mutations in the histone H3 gene family, we performed targeted sequencing of the exons encompassing K27 from all 16 genes encoding histone H3 isoforms in a validation cohort containing 43 DIPGs, as well as 36 non-brainstem paediatric glioblastomas (non-BS-PGs) (see Supplementary Methods, Supplementary Tables 1 and 2). Including the original 7 DIPG patients analyzed by WGS, we found recurrent A to T transversions encoding p.K27M mutations in *H3F3A* or *HIST1H3B* in 78% of DIPG, and 22% of non-BS-PGs (Table 1, Figure 1). In addition, a novel guanine to adenine transition resulting in p.G34R mutation in *H3F3A* was identified in 5/36 (14%) of non-BS-PGs, but not in any of 50 DIPGs analyzed. These three different H3 mutations were mutually exclusive. There was no evidence of loss of heterozygosity at the mutant loci by WGS and SNP array in the discovery cohort, and by Sanger sequence and SNP array in the validation cohort. For 32 DIPG samples and six non-BS-PG samples in which an *H3F3A* or *HIST1H3B* mutation was identified and matched normal DNA was available, the germline DNA was wild-type, verifying that these mutations were somatic (Supplementary Table 1). Importantly, the p.K27M alterations were found in seven of eight DIPG samples obtained prior to therapy, indicating that this alteration was not secondary to therapy-induced mutagenesis. There were no K27 or G34 mutations in any of the other 14 genes encoding histone H3 variants.

The identified alterations in histone H3 appear to be exclusive to paediatric high-grade gliomas. We also detected *H3F3A* p.K27M mutation in one of 9 paediatric anaplastic astrocytomas (Grade III), but no mutations in any of the 16 histone H3 genes were detected in other paediatric brain tumours including 7 low-grade brainstem gliomas evaluated by targeted Sanger sequencing, or in whole genome sequence data from 15 low-grade non-brainstem gliomas, 38 medulloblastomas, and 22 ependymomas, nor in an additional 170 non-central nervous system paediatric tumours (Supplementary Tables 1 and 3). We also did not find any evidence of structural variations in the histone H3 loci in any of the tumour subtypes evaluated by whole genome sequencing. There were also no occurrences of K27 or G34 germline polymorphisms in any of the histone H3 genes in dbSNP 135 (http://www.ncbi.nlm.nih.gov/projects/SNP/), which includes 39,484,957 SNPs submitted by 1000 Genomes Project or the variants identified by NHLBI ESP 5400 exomes project (http://evs.gs.washington.edu/EVS/). In fact, no non-silent coding germline variants were found in *H3F3A* and only one (p.Q20E) in *HIST1H3B* in these two public SNP databases.

H3F3A is located on chromosome 1q, a region of large-scale chromosomal gain in more than 20% of both DIPGs and non-BS-PGs[2–8], however there was no significant correlation

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between the presence of *H3F3A* or *HIST1H3B* mutations and gain of chromosome 1q (p=0.7). There was also no significant association between *H3F3A* or *HIST1H3B* mutation and amplification of receptor tyrosine kinases (p=0.7), or amplification of CyclinD family genes, or CDK4 or CDK6, (p=0.5), alterations that occur in greater than 30% of DIPGs[2,4,6].

Although it is unclear exactly how these mutations alter H3 function, H3K27 and H3G34 are within the highly conserved N-terminal tail of histone H3, which influences the dynamic regulation of chromatin structure and accessibility. Alterations at these invariant residues, which are close to the site where the tail exits the globular histone core of the nucleosome, may affect nucleosome structure and function by impacting histone-DNA interactions, chromatin compaction, or interactions with other effectors that bind to histones. Complex posttranslational modifications of the histone tail play an important role in epigenetic regulation of gene expression by affecting both chromatin state and direct interactions between modified histones and transcriptional activator or repressor complexes [9]. It is notable that trimethylation of H3K27 is associated with silencing genes, especially those involved in developmental processes in pluripotent cells, and monomethylation is associated with gene activation[10]. Acetylation and deacetylation of H3K27 are also highly regulated, with acetylated K27 associated with transcriptionally active regions [11]. Replacement of K27 with methionine implies loss of function, as it removes the ability to methylate or acetylate this position, which may impact its role in either repression or activation. However, the mutations are present in the heterozygous state in only two of a large family of H3 genes, and always encode the same amino acid substitutions, suggesting a gain-of-function. Additional studies are required to determine the functional impact of p.K27M and p.G34R alterations.

Importantly, mutations were found in more than one histone H3 variant. H3.1 and H3.2 are replication-dependent histones, synthesized and incorporated into nucleosomes during S-phase, while H3.3 is a replication-independent variant that is selectively enriched within actively transcribed genes, transcription factor binding sites, and at telomeres where its incorporation can affect telomere stabilization [12]. The higher frequency of *H3F3A* mutations suggests that disruption of the specialized functions of H3.3 may provide a specific selective advantage. However, the recurrent and mutually exclusive p.K27M monoallelic substitutions in either *H3F3A* or *HIST1H3B* suggest a similar gain of function effect is possible, despite the differences in regulation of H3.3 and H3.1. It is possible that differential transcriptional or posttranslational regulation of *H3F3A* and *HIST1H3B* relative to other H3 variants, may underlie in part why these two genes are selectively targeted by mutation in the cells of origin for DIPG and non-BS-PGs.

Based on the complex regulatory roles of histone H3, these mutations could potentially impact epigenetic regulation of gene expression, selective regulation of developmental genes, or telomere length or stability. Although mutations in genes encoding posttranslational modifiers of histones have been identified in a number of different cancers [13], this is the first report of somatic mutations in histone H3. Perhaps consistent with a role for H3K27 in regulating expression of genes associated with development, histone H3 mutations were not identified in exome sequence data from adult glioblastomas [14]. This indicates that these mutations confer a selective advantage in the unique context of

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developing brain, and highlights the significant difference in the underlying biology of gliomagenesis in children and adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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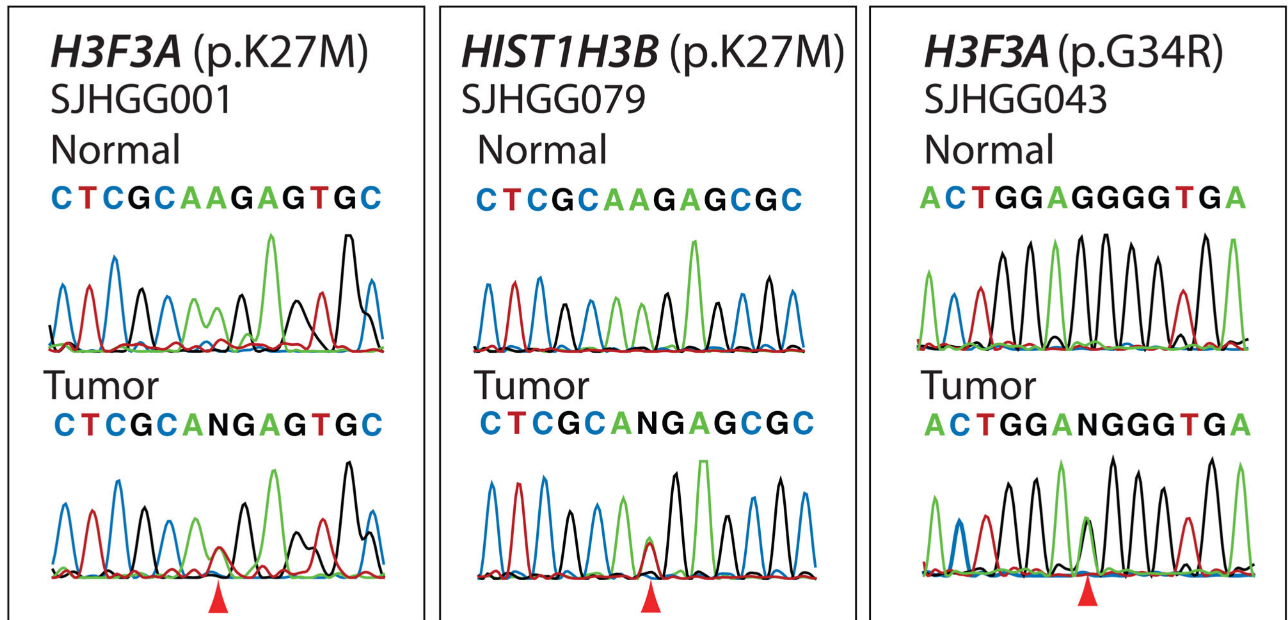

Figure 1. Recurrent somatic mutations in *H3F3A* and *HIST1H3B*

Sanger sequencing chromatograms showing representative *H3F3A* or *HIST1H3B* mutations encoding p.K27M substitutions, or *H3F3A* mutation encoding p.G34R substitution in the indicated tumour compared to matched normal DNA. Arrow indicates mutation. SJHGG001 and SJHGG079 are DIPGs, SJHGG043 is a non-brainstem paediatric glioblastoma.

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Table 1

Frequency of recurrent somatic mutations in diffuse intrinsic pontine gliomas (DIPG) and non-brainstem paediatric glioblastomas (GBM)

Gene	AA Change	DIPG (n=50)	GBM (n=36)
H3F3A	p.K27M	30 (60%)	7 (19%)
H3F3A	p.G34R	0	5 (14%)
HIST1H3B	p.K27M	9 (18%)	1 (3%)
All H3		39 (78%)	13 (36%)