Gene of the month: H3F3A and H3F3B

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Accepted 1 November 2021
Published Online First 15 November 2021

ABSTRACT

H3F3A and *H3F3B* genes are located at 1q42.12 and 17q25.1, respectively, and encode identical H3.3 core histone proteins which form part of the histone hetero-octamer complex. Histones function by packaging DNA into small units, the nucleosome, and are highly susceptible to epigenetic post-translational modification. H3 K27 mutations have been shown to inhibit the polycomb repressive complex 2, which is normally involved in epigenetic gene silencing. Mutations in *H3F3A* and *H3F3B* are increasingly recognised in a variety of solid tumours. Point mutations in *H3F3A* have been described in giant cell tumour of bone and paediatric-type diffuse high-grade gliomas. Mutations in *H3F3B* have been described in chondroblastoma. Loss of trimethylation of H3 K27 is characteristic of most sporadic and radiation-associated malignant peripheral nerve sheath tumours. Immunohistochemistry with a variety of novel antibodies directed against specific mutations, as well as loss of H3K27me3 staining, may be useful in specific settings and in diagnostically challenging cases.

INTRODUCTION

Histones are nuclear proteins that maintain the nucleosome structure of chromatin in eukaryotic organisms.[1] H3 is one of the four core histone proteins which comprise the histone hetero-octamer, around which approximately 147 base pairs of DNA wrap to form the nucleosome.[2]

GENE AND PROTEIN STRUCTURE

In mammals, the H3 family includes the canonical replication-dependant H3.1 and H3.2, and the replication-independent variant H3.3.[3] *H3F3A* and *H3F3B* encode identical conserved H3.3 histone proteins (figure 1) which differ only in their mRNA untranslated regions and regulatory sequences.[4] *H3F3A* is located on chromosome 1q42.12 and comprises seven exons.[5] *H3F3B* occurs on chromosome 17q25.1 and comprises four exons.[5] Both *H3F3A* and *H3F3B* genes contain introns and their mRNAs and are polyadenylated, unlike the genes encoding the canonical H3.1 and H3.2 histone proteins.[6] The H3.3 protein consists of a 136-amino acid sequence with a molecular weight of 15.328 kDa.[7] The structure of the core histones is predominantly globular, other than their N-terminus tail portions which are unstructured. The histone hetero-octamer consists of a tetramer H3–H4 complex flanked by two H2A–H2B dimers.

PHYSIOLOGICAL FUNCTION

Histones compact DNA into the fundamental unit of chromatin, the nucleosome, while still enabling the cellular processes operating on DNA to occur. These processes include transcription, DNA repair, DNA replication and chromatin condensation. Histone function is modulated by at least eight covalent post-translational modifications including acetylation, phosphorylation and methylation.[8] Another manner by which nucleosomes are controlled is the incorporation of histone variant proteins into the hetero-octamer complex. Histone variant H3.3 differs from the canonical H3 proteins (H3.1 and H3.2) in that its incorporation onto chromatin is cell cycle independent (ie, occurring throughout the cell cycle and not S-phase dependent) and mediated by the chaperone complexes histone regulator A (HIRA) and the death-associated protein (DAXX)/α-thalassaemia X-linked mental retardation protein (ATRX).[9 10] H3.3 is enriched in genomic regions showing 'active' transcription, pericentromeric and telomeric regions.[6 11] H3 K27 mutations have been shown to inhibit the polycomb repressive complex 2 (PRC2), which is normally involved in epigenetic gene silencing.[12]

ROLE IN DISEASE

Mutations in *H3F3A* and *H3F3B* genes are increasingly recognised in a variety of tumours ranging from indolent bone tumours to highly aggressive brain tumours (table 1).

Chondroblastoma

Chondroblastoma is an uncommon benign tumour of bone, typically occurring in skeletally immature long bones. The femur, proximal tibia and proximal humerus are the sites most commonly affected. The age range is 10–25 years and males are twice as likely to be affected.[13] These tumours occur adjacent to the growth plate, typically involving the epiphysis or epiphysis and metaphysis as well-defined radiolucent lesions on plane film imaging.[14] Histologically, these tumours are composed of sheets of chondroblastic cells with distinct cell borders, grooved nuclei and dispersed osteoclast-like giant cells.[15] Pericellular chicken-wire calcification is a distinctive feature. In the vast majority of cases, these tumours are cured by surgical curettage.[16] Recurrence rates vary with the site of disease and are higher in flat and craniofacial bones.

Behjati *et al* were the first to identify the lysine amino acid substitution p.Lys36Met in 73 of 77 chondroblastomas studied.[17] This point mutation occurred predominantly in *H3F3B* but was also found in a small proportion of cases to occur in *H3F3A*. These findings have been subsequently

To cite: Aldera AP, Govender D. *J Clin Pathol* 2022;**75**:1–4.

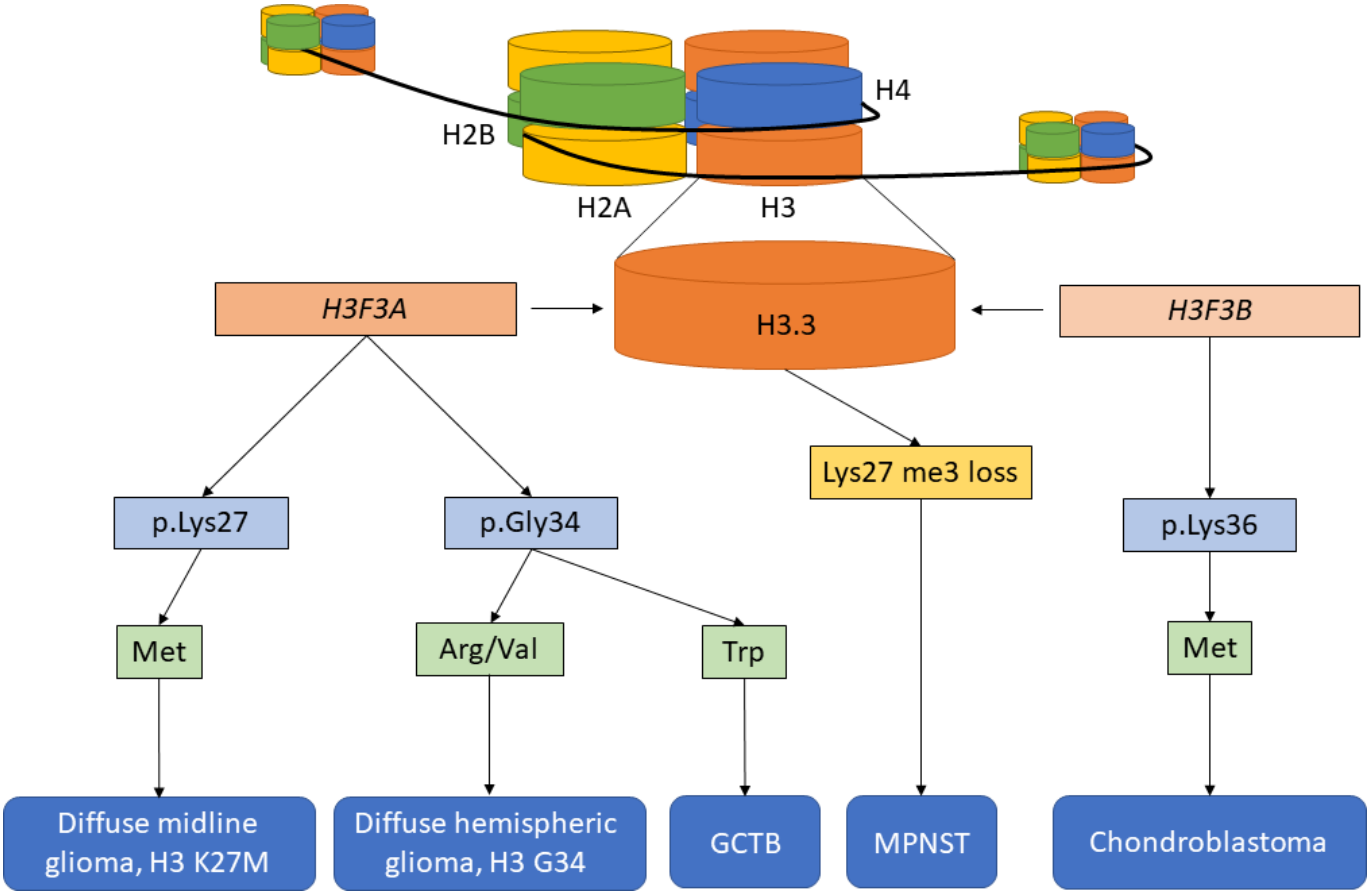

Figure 1 *H3F3A* and *H3F3B* genes encode identical H3.3 proteins. Point mutations at key amino acid residues give rise to diverse tumours. This infogram highlights the most common point mutations and associated tumours. GCTB, giant cell tumour of bone; MPNST, malignant peripheral nerve sheath tumour.

confirmed by other groups.[18] Immunohistochemistry with the H3 K36M mutant antibody shows nuclear positivity in >96% of cases and is of particular value in limited biopsies or cases with extensive aneurysmal bone cyst-like change.[19–21]

Giant cell tumour of bone (GCTB)

GCTB is a locally aggressive and rarely metastasising neoplasm which has a similar anatomical distribution to chondroblastoma but typically occurs in skeletally mature individuals. The peak incidence is between the ages of 20 and 45 years.[22] Microscopically, GCTB comprises sheets of mononuclear cells which may be round to oval in shape, or spindled when associated with a fibrous matrix, with conspicuous numbers of osteoclast-like giant cells. Secondary changes may obscure the typical morphology and make diagnosis more challenging. These include necrosis, fresh haemorrhage, collections of macrophages, haemosiderin deposition, aneurysmal cyst change and deposition of fibrous tissue or bone. GCTB may metastasise to the lung. Malignant GCTB is uncommon and characterised by a nodule of pleomorphic mononuclear cells occurring in an otherwise conventional GCTB. GCTB treated with the monoclonal antibody targeting the key bone resorption mediator RANKL, denosumab, may show abundant new bone formation, bland spindled cells and depletion of the osteoclast-like giant cells.[23]

Approximately 95% of GCTBs harbour recurrent mutations in *H3F3A*. These are most commonly amino acid substitutions involving a glycine residue (p.Gly34Trp). Less common point mutations that have been described include p.Gly34Leu, p.Gly34Met, p.Gly34Arg and p.Gly34Val. Immunohistochemistry with a monoclonal antibody directed against the H3 Gly34Tryp point mutation (G34W) has a high sensitivity and specificity.[24 25] Yamamoto *et al* found H3 G34W expression in all the recurrent, metastatic, post-treatment (denosumab) and secondarily malignant GCTB.[25]

Table 1 Tumours associated with H3.3 aberrations

	Mutation	Gene	Immunohistochemistry
Chondroblastoma	p.Lys36Met	*H3F3B* (95%) *H3F3A* (5%)	H3 K36M
Giant cell tumour of bone	p.Gly34Trp	*H3F3A*	H3 G34W
Diffuse midline glioma, H3 K27-altered	p.Lys27Met	*H3F3A* *HIST1H3B/C*	H3 K27M
Diffuse hemispheric glioma, H3 G34-mutant	p.Gly34Arg/Val	*H3F3A*	H3 G34R/V
Malignant peripheral nerve sheath tumour	Loss of trimethylation	*EED* *SUZ12*	H3K27me3 (loss)

Paediatric-type diffuse high-grade gliomas

Diffuse midline glioma, H3 K27-altered, is defined as a high-grade infiltrative neoplasm which occurs in the midline, shows predominantly astrocytic differentiation and harbours distinctive mutations in H3. This is the new terminology proposed in the upcoming fifth edition WHO Blue Book as it has become

increasingly clear that alternative mechanisms can result in alteration of the pathogenic pathway in these tumours.[26] Historical designations have included 'brainstem glioma' or 'diffuse intrinsic pontine glioma'. These tumours occur predominantly in children and have a very poor prognosis. The 2-year survival is less than 10% despite current best therapies. In these tumours, the traditional morphological features used to grade glial neoplasms (mitoses, necrosis and microvascular proliferation) are not reliable. Sequencing studies have identified the recurrent heterozygous mutation p.Lys27Met (K27M) affecting *H3F3A* or *HIST1H3B/C* (encoding H3.1).[27] Mutations in *H3F3A* are about three times as prevalent as those in *HIST1H3B/C*.

H3 K27M immunohistochemistry can be used to identify tumours harbouring K27M mutations with high sensitivity and specificity and minimal intratumoural heterogeneity.[28] Nuclear staining is regarded as positive, and care must be taken to disregard non-specific cytoplasmic staining, which may be seen in macrophages and glial cells. The H3K27me3 antibody (decreased protein expression interpreted as a surrogate marker for H3 K27M) may be used as a screening method to identify cases, but interpretation is often challenging due to non-specific staining of inflammatory cells, endothelial cells and glial tissue.[28]

Diffuse hemispheric glioma, H3 G34-mutant (DHG) is a newly recognised brain tumour which typically occurs in the cerebral hemispheres of teenagers and young adults, and generally has a more favourable prognosis than glioblastoma, isocitrate dehydrogenase (IDH)-wildtype and diffuse midline glioma, H3 K27-altered.[29] This is still, however, a WHO grade 4 tumour. Histologically, these tumours are heterogeneous and may resemble high-grade gliomas or primitive neuroepithelial tumours. DHG is genetically defined by a recurrent point mutation, p.Gly34Arg/Val (G34R/V), occurring in *H3F3A*. Mutant-specific antibodies for H3 G34R and H3 G34V are commercially available and have been found to show a high degree of sensitivity and specificity for DHG.[30]

Malignant peripheral nerve sheath tumour

Malignant peripheral nerve sheath tumour (MPNST) is a malignant soft tissue neoplasm which may arise de novo from a pre-existing benign nerve sheath tumour or in the setting of neurofibromatosis type 1. These tumours have complex karyotypes and frequently show combined inactivation in three pathways *NF1*, *CDKN2A*/*CDKN2B* and PRC2 core components (embryonic ectoderm development (*EED*) and suppressor of zeste 12 (*SUZ12*)).[31 32] Mutations in *EED* and *SUZ12* induce loss of trimethylation at lysine 27 of histone 3 (H3K27me3).[33] Outside of the setting of NF1, the diagnosis of MPNST is often challenging with high interobserver variability and relies on a combination of morphological features, patchy expression of S100 or SOX10 and the demonstration of possible involvement of a nerve structure.

Complete or partial loss of H3K27me3 nuclear expression by immunohistochemistry is highly specific for MPNST.[34 35] The sensitivity of this marker is somewhat modest and almost comparable to staining with S100 protein, ranging from 57% to 69%. Fortunately, the sensitivity is higher in sporadic cases and those associated with radiotherapy (91%–95%), where the diagnosis is often more challenging.[34] Heterozygous staining or partial loss is demonstrated in a small proportion of cases. Interestingly, loss of H3K27me3 is less useful diagnostically in the setting of NF1 occurring in only 60% of cases. Of note, H3K27me3 expression is retained in epithelioid MPNST.

Developmental and behavioural disorders

In animal studies, knockout of H3.3 has shown its important role in neurodevelopment and neuromuscular function.[36] This has not yet been thoroughly investigated in humans. Recently, a group from Slovenia who performed whole exome sequencing on a patient with microcephaly and developmental delay found a missense variant c.185 T>G in *H3F3A*.[37]

Take home messages

- *H3F3A* and *H3F3B* encode identical non-canonical variant H3.3 proteins which form part of the histone hetero-octamer, around which approximately 147 base pairs of DNA wrap to form the nucleosome.
- Histones function to maintain the structure of chromatin and are influenced by a variety of post-translational modifications.
- Point mutations in *H3F3A* have been described in giant cell tumour of bone and paediatric-type diffuse high-grade gliomas, while point mutations in *H3F3B* have been described in chondroblastoma.
- Loss of trimethylation of H3 K27 is characteristic of most sporadic and radiation-associated malignant peripheral nerve sheath tumours.
- Immunohistochemistry with antibodies directed against specific mutations as well as H3K27me3 are useful diagnostically.

Handling editor Runjan Chetty.

Contributors The manuscript was prepared by both authors.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not applicable.

Provenance and peer review Commissioned; internally peer reviewed.

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