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Review

Histone Modification in Histochemistry and Cytochemistry

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Keeping chromatin in a stable state is essential for genome stability, scheduled transcription, replication, DNA repair, and precise and reliable chromosome segregation and telomere maintenance during cell division. Over the past decade, research on chromatin remodeling has made great strides whereby modification of histone proteins is a key factor involved in many of the essential cellular processes. The nuclear findings of tumor cells that pathologists routinely examine are nothing but reflections of both genomic and histone alterations. Moreover, impaired histone function is known to be related to common diseases such as diabetes and atherosclerosis, and is, therefore, considered a potential therapeutic target. The present review first outlines the physiological function of histone proteins, and second, demonstrates their alterations to pathological states, emphasizing the importance of immunohistochemistry in histopathological diagnosis.

Key words: epigenetics, DNA methylation, histone modification, immunohistochemistry, histopathology

I. Functional Significance of Histone Modification

Chromatin is a complex of nuclear DNA and histone protein, and DNA is compactly folded and highly compressed by wrapping around histone protein. Since 20% or more of the constituent amino acids of histones are basic residues—lysine and arginine—they can strongly bind to the sugar-phosphate backbone of the negatively charged DNA chain. “Nucleosomes” are the basic structure of chromatin where 147 base pairs of double helix DNA are wound around histone proteins, and the DNA that connects nucleosomes is termed linker DNA. As a result of densely arranged nucleosomes that condense chromatin by repeatedly folding multiple stages, the length of the chromosome during mitosis shrinks to 1/10,000 of the original DNA. Higher-order chromatin formation, however, does not merely shrink the DNA [13]. The chromatin structure varies dynamically even on interphase chromosomes, with highly condensed heterochromatin regions and loosely cohesive euchromatin regions coexisting to select DNA transcription, replication, and repair sites in the cell nucleus (Fig. 1a). Heterochromatin occupies about 10% of interphase chromosomes and is particularly concentrated in the centromere and near telomere regions, where various nucleoproteins do not approach DNA, and thereby gene transcription is rarely induced. To the contrary, in the euchromatin region, the packing is loose, enabling effector proteins to approach the DNA, resulting in active transcription (Fig. 1b). These two chromatin states being mutually exchangeable, enable spacial and sequential gene transcription [10].

The nucleosome, is the basic structure of chromatin, comprising 147 base pairs of DNA wrapped around an octamer containing two spherical core histones H2A, H2B, H3, and H4 [5]. The octamer is bundled by histone protein H1 and linked to the next nucleosome (Fig. 2a). The core histone protein consists of a globular domain and N-

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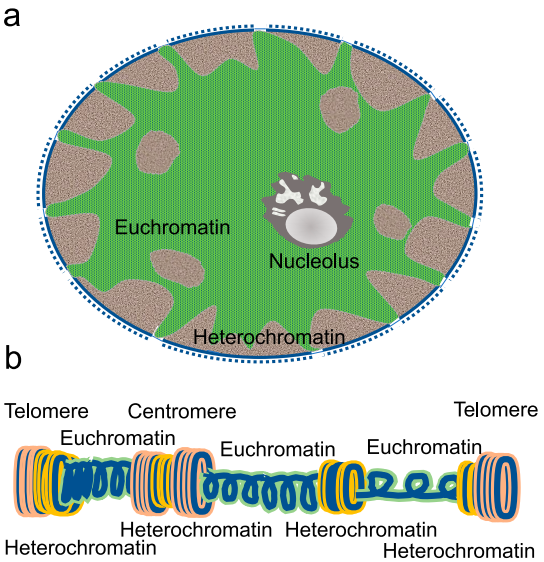

Fig. 1. (**a**) Schematic diagram of the nucleus. Heterochromatin shown dark is distributed in the margins near the nuclear membrane, while euchromatin is distributed in the bright areas. (**b**) Chromatin structure of interphase chromosome. Highly condensed heterochromatin is concentrated near the centromere and telomere. In other regions, condensed and loosened chromatin is variable.

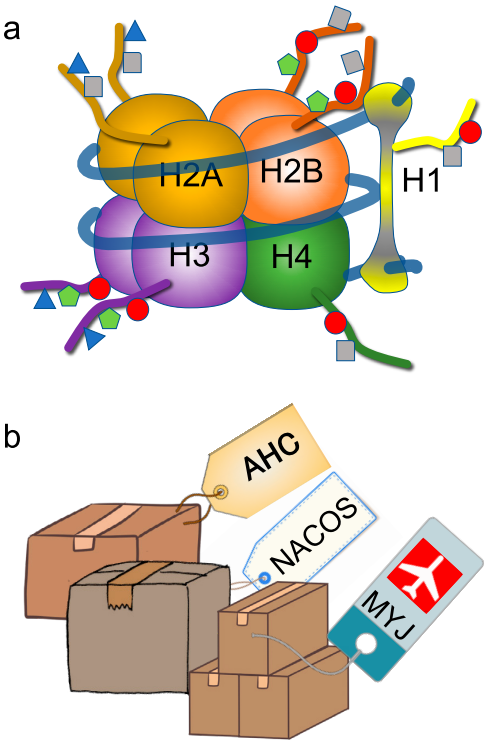

Fig. 2. (**a**) Schematic diagram of the nucleosome. Histones H3 and H4 form H3-H4 dimer, and two H3-H4 dimers form a tetramer, to which two H2A-H2B dimers are added to form a histone octamer. The histone octamer consists of two each of H2A, H2B, H3, and H4. 147 base pairs of DNA are wrapped around the octamer and packed bead-like. In the doorway of DNA, linker histone H1 attracts the next nucleosome and assists in chromatin aggregation. Histone proteins consist of a globular domain and N- and C-terminal tails, and post-translational modifications such as methylation, acetylation, and phosphorylation are added to specific amino acid residues of the N-terminal tail. (**b**) The modification of the N-terminal tail of histone proteins serves as a luggage tag that identifies the "destination" or "handling" of the protein. It is important that tags are properly written and read, since loss, damage, or misdescription of tags can cause confusion and damage.

terminal and C-terminal tails, which are acetylated (acetylation [ac]), phosphorylated [ph], and methylated by post-translational modification (PTM). Phosphorylation (methylation [me]) and ubiquitination (ubiquitination [ub]) are added to specific amino acid residues. Since acetylation and ubiquitination are on lysine (lysine [K]), methylation is on lysine and arginine (arginine [R]), and phosphorylation is on serine (serine [S]) and threonine (threonine [T]) residues, the lysine [K] site undergoes either acetylation or methylation modification. Since the N-terminal tail with such modification protrudes from the nucleosome, it serves as a "modification code" like a baggage tag, serving as a marker for various nuclear proteins to approach DNA and activate transcription (Fig. 2b).

Unlike the heterochromatin region, histone acetylation such as H3K9ac and H4K16ac is observed in the euchromatin region, and the acetylation modification is generally associated with the transcriptional activity of the gene. When an acetyl group is added by the histone acetyltransferase (HAT), the binding between histones and DNA changes due to a decrease in the positive charge, and thereby the aggregation of nucleosome structures is loosened, enabling RNA polymerase to bind to the transcription start site. Conversely, removal of acetyl groups by histone deacetylase (HDAC) strengthens the binding between histones and DNA and leads to the suppression of gene expression. The significance of methylation modification is, however, not simple. In the region of high transcriptional activity, histone H3 has lysine 4 (K4) methylated, but dimethylation is present in both the highly active and inactive states, and trimethylation is present only in the active gene [20]. Moreover, while H3K4 methylation acts on transcriptional activation, H3K9 methylation is involved in gene inactivity [1, 12, 19]. This complexity—coexistence of "promotion" and "suppression"—by methylation modification is explained in that, unlike acetylation and phosphorylation, it does not cause an obvious change in the positive charge. Furthermore, methylations like H3K36me3 have unique distribution showing a peak toward the 3'-end of the transcribed gene, and are considered to be involved in transcription termination and early RNA processing [2]. In addition, H3K27ac and this H3K4me1 are known to act as transcription promotion markers, and H3K9me3 and H4K20me3 are known to act as transcription suppression markers (Fig. 3a).

Histone modifications are not immobilized, and methylation added by histone methyltransferase (HMT) is removed by histone demethylase (HDM). Methylation modification functions as a dynamic and reversible marker

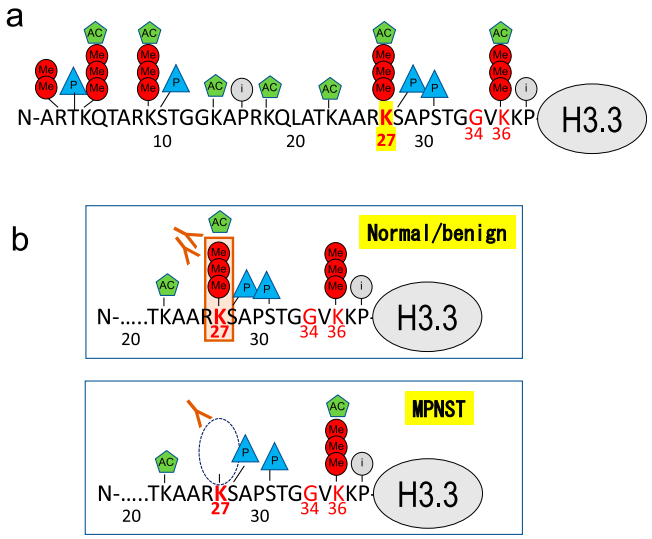

Fig. 3. (**a**) Amino acid sequence of the N-terminal tail and modification sites of histone 3.3. Highly mutated sites (K27, G34, K36) are shown in red. Me: methylation, ac: acetylation, p: phosphorylation, i: isomerization. (**b**) H3K27me3 is an inhibitory chromatin modifier in which lysine 27 is trimethylated, and the polycomb repressive complex PRC2 is involved in its methylation. In malignant peripheral nerve sheath tumor (MPNST), H3K27 is not methylated due to mutations in the PRC2 components EED and SUZ12. In normal tissue and benign schwannoma, trimethylation is detected by anti-H3K27me3 antibody, but is negative in MPNST.

due to the action of numerous post-translational modification enzymes termed "Writer" and "Eraser". The significance of histone modification is to control the binding of the "Reader" protein complex that reads the determined modification and to provide a scaffold for the complex responsible for chromatin structural conversion to function.

During chromatin condensation, K27 of histone H3 is methylated by the methyltransferase enhancer of zeste homologue (EZH) 1/2 that is the main component of polycomb repressor complex 2 (PRC2) [8]. H3K27me3 serves as a marker for the region where transcription is silenced, and the PRC1 complex binds to this region to promote chromatin compression. When relaxing chromatin, on the other hand, the chromatin remodeling complex uses the energy gained from the hydrolysis of ATP to locally move the position of the DNA wrapped around the nucleosome. This is the SWI/SNF (switch/sucrose non-fermenting) family or BAF complex (Brg/Brm-related factor), and binds to acetylated lysine residues. Gene mutations in the components of the BAF complex are involved in many tumors, especially in bone and soft tissue ones [17].

II. Variants and Mutations of Histone Proteins

Histones have subtypes collectively termed histone variants. As listed in Table 1, many of the histone variants are localized in the testis. When sperm in mammals undergoes nuclear aggregation, 90% or more of histone proteins are replaced with protamine, resulting in mature sperm. Some histone variants are necessary at this aggregation stage, and tissue localization of such variants is naturally seen in the testis. While the impaired function of these spermatogenesis-related histone variants may affect spermatogenic potency, its association with tumorigenesis and tumor progression is also considered to be low. On the other hand, histone variants directly involved in chromatin regulation are known to be involved in many tumors [4, 11, 16, 18, 22]. Hereditary diseases caused by germline abnormalities of genes related to these histone variants themselves are limited to a few rare diseases such as Floating-Harbor syndrome and Nijmegen breakage syndrome (Ataxia-telangiectasia variant V1).

For individual histone variants unrelated to the spermatogenesis, except for H2A.X participating in the DNA repair process, most of the variants are involved in regulation of gene expression. Constitutively and globally expressed H4 histone had been assumed to have no variants until the recent identification of a human-specific variant, H4G, found in many tumor cell types [14]. Furthermore, a fifth histone, the linker histone H1 (not listed in Table 1), which had received little attention and termed the "forgotten histone", has also been shown to undergo various post-translational modifications and to yield functioning variants. In recent years, changes in the composition, expression level, and point mutations of histone H1 variants have been demonstrated in many types of cancer, and are the subject of future research [6].

The variants of histone H3, H3.1 and H3.2 are expressed only in the S phase in a DNA replication-dependent manner. H3.3 comprises two genes, H3F3A and H3F3B, and is expressed throughout the cell cycle. H3.3 is located not only in transcriptionally active euchromatin regions but also in pericentromere and telomere regions as well as in heterochromatin loci. Since H3.3 accounts for nearly 90% of the total H3, the impact of genetic alterations of H3.3 is large especially in relation to tumorigenesis (Fig. 3a and b).

III. Tumor Histopathological Diagnosis and Histone Protein Abnormalities

Six histone H3.3 mutations, K27M, G34R/V, G34W/L, and K36M, have been demonstrated at three locations, K27, G34, and K36 [15]. For validating histopathological diagnosis, number of tumors identifiable immunohistochemically with the use of specific antibodies has been increasing.

Glioma: with antibodies H3K27M and G34R/V

Mutations H3K27M and G34R/V in the H3F3A gene encode histone variant H3.3. In particular, mutation H3K27M is present in about 80% of high-grade gliomas in children, but it is a significantly poor prognostic factor [7, 21]. Diffuse intrinsic pontine glioma (DIPG) is an infiltra-

Table 1. *Core histone variants, their function and relation to disease*

Core histone	Tissue distribution	General function	Dysregulation and role in malignancies		Associated genetic disorders
H2A family					
H2A.Z.1	Global	Binding of regulatory complexes and chromatin dynamics	Amplification and missense mutations	oncogenic	Floating-Harbor syndrome
H2A.Z.2	Global	Binding of regulatory complexes and chromatin dynamics	Amplification and missense mutations	oncogenic	Floating-Harbor syndrome
macroH2A1 macroH2A2	Global	Gene silencing and higher-order chromatin compaction	Transcriptional repression and splicing defects and amplification	suppressor	ND
H2A.X	Global	DNA damage response and chromatin remodeling	Mutation or deletion	suppressor	Nijmegen breakage syndrome
H2A.B	Testis and brain	Nucleosome destabilization, and active transcription and mRNA splicing	ND	ND	ND
H2A.L	Testis	Histone-to-protamine transition	ND	ND	ND
H2B family					
TH2B (TS H2B.1)	Testis	Histone-to-protamine transition	ND	ND	ND
H2B.W (H2BFWT)	Testis	ND	ND	ND	ND
H3 family					
H3.3 HIRA-UBN-CABIN1	Global	Transcriptional activation and chromatin dynamics	Amplification and missense mutations	oncogenic	ND
H3.3 ATRX-DAXX	Global	Heterochromatin formation and telomere stabilization	Missense mutation	suppressor	α-Thalassaemia X-linked mental retardation syndrome
H3.Y.1 H3.Y.2 (H3.X)	Testis and brain	Transcriptional activation	ND	ND	ND
CENP-A	Global	Centromere identity and genome stability	Amplification or overexpression	Chromosome missegregation and chromosome instability	ND
H3.4 (H3T)	Testis	Histone-to-protamine transition	ND	ND	ND
H3.5	Testis	Histone-to-protamine transition	ND	ND	ND
H4 family					
H4G	Global	Upregulation of rDNA transcription	ND	oncogenic	ND

In addition to the diversity of core histones due to PTMs, numerous variants are present. Most of the histone variants required for spermatogenesis are localized in the testis. On the other hand, alterations of the variants directly involved in chromatin regulation are known to be involved in tumorigenesis and tumor progression.
Modified from Refs. [14] and [19].

tive astrocytoma arising in the median region of the brain, especially in the thalamus and the brainstem, with histological features corresponding to not only diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma, but also tumor-like components, presenting a complex mixture of various histological features. H3K27M, an important marker for confirming the pathological diagnosis of DIPG, is an alteration from lysine 27 to methionine in H3.3. H3K27 is a critical portion in that it is modified by acetylation as well as by mono-, di- and tri-methylation, and is a target site of polycomb repressive complex 2 (PRC2) transcriptional repressor. Alteration of this critical site from lysine 27 to methionine causes the loss of its transcriptional repressive marker, leading to transcriptional dysregulation of various genes and promoting cell growth and transformation, which in turn promote tumor formation [15].

Malignant peripheral nerve sheath tumor (MPNST): with antibodies H3K27me3

Since H3K27me3 is absent from or decreased in MPNST, it differentiates MPNST from other histologically similar diseases. Benign Schwannoma specimens (Fig. 4)

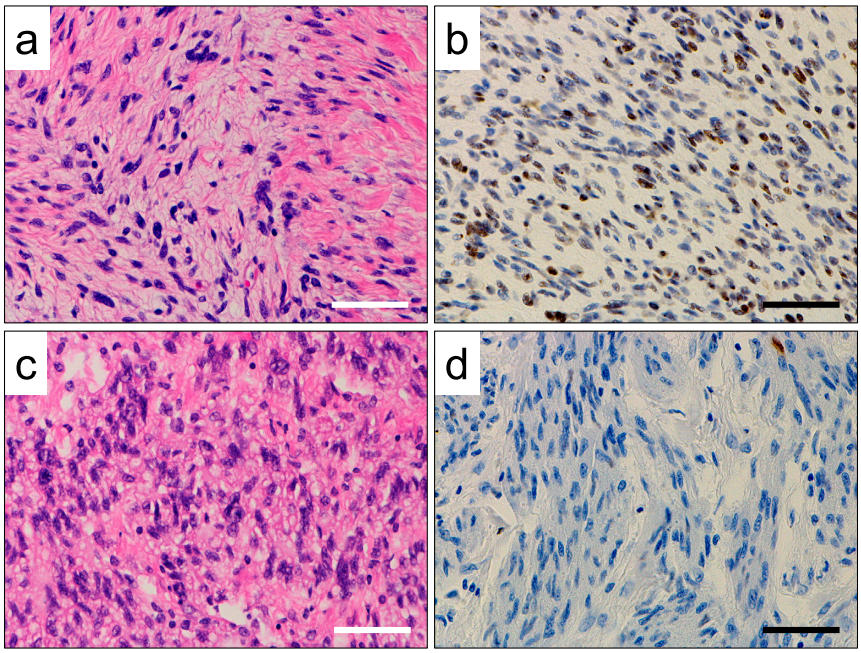

Fig. 4. Histological analysis of malignant peripheral nerve sheath tumor (MPNST) tissue sections using anti-H3K27me3. (**a**, HE stain) Resected specimen of benign schwannoma. Proliferation of Schwann cell-like spindle-shaped cells is observed. (**b**) Immunohistochemical staining with anti-H3K27me3 antibody. In schwannoma, more than half of the tumor cells are positive in their nuclei. (**c**, HE stain) Resected specimen of MPNST. Dense proliferation of spindle-shaped cells is observed. (**d**) Immunohistochemical staining with anti-H3K27me3 antibody. MPNST tumor cells are negative. Bars = 100 μm.

displayed proliferation of schwann cell-like spindle-shaped cells (Fig. 4a, HE stain), and by immunohistochemical staining with anti-H3K27me3 antibody, more than half of the tumor cells were present in their nuclei (Fig. 4b). On the other hand, while resected specimens of MPNST displayed dense proliferation of spindle-shaped cells similar to those of the benign Schwannoma (Fig. 4c, HE stain), immunohistochemical staining with anti-H3K27me3 antibody revealed total absence of tumor cells in their nuclei (Fig. 4d). As mentioned above, H3K27me3 is a repressive chromatin modification in which lysine 27 of the histone protein is trimethylated, and the polycomb repressive complex PRC2 is involved in the methylation. In MPNST, H3K27 methylation is lost due to mutations of PRC2 components in EED and SUZ12 (Fig. 3b) [23].

Giant cell tumor of bone (GCTB): with H3G34W antibodies

Giant cell tumor of bone occurs most often in the metaphysis of the femur and tibia in individuals in their 20s to 40s, and accounts for 5–10% of primary bone tumors. H3.3 encoded by the H3F3A gene, mutates from 34 glycine to tryptophan (G34W) but rarely to leucine (G34L) [3]. In GCTB, the osteoclast differentiation factor RANKL produced by mononuclear tumor cells induces osteoclast-like multinucleated giant cells, resulting in the formation of prominent osteolytic lesions that appear on diagnostic imaging. In histological studies, the prominent osteolytic lesion in GCTB needs to be differentiated from secondary aneurysm-like bone cysts and giant cell-repairing granulomas. Immunostaining with the H3G34W antibody confirms the diagnosis of GCTB. Recently, Denosmab, the RANKL neutralizing antibody that suppresses bone resorption has been used as a therapeutic agent for GCTB. The histological image of GCTB often changes after Denosmab administration. In the resected tumor tissue before (Fig. 5a, HE and b, immunohistochemical staining with anti H3G34W antibody) and after Denosmab administration (Fig. 5c, HE and d, immunohistochemical staining with anti H3G34W antibody), while osteoclast-like giant cells (surrounded by dashed lines) remarkably decreased after Denosmab treatment, the novel cell type, spindle-shaped osteoblast-like cells (OS), proliferated and formed vigorous osteoid and young woven bone (Fig. 5). H3.3G34W-positive immunostaining of these novel proliferating osteoblast-like cells confirms that it maintains the original somatic mutation of GCTB after a dramatic morphological transformation. Since G34 is located near the doorway of nucleosomal DNA and in close proximity to lysine K36, by replacing glycine at position 34 with tryptophan of a large molecular size, it is speculated that the binding to SET domain contains 2 histone lysine methyltransferases (SETD2). When the binding of SETD2, a transcriptional regulator acting on K36, is inhibited, K36 trimethylation (H3K36me3) by SETD2 is consequently lost, resulting in alteration of transcriptional regulation [9].

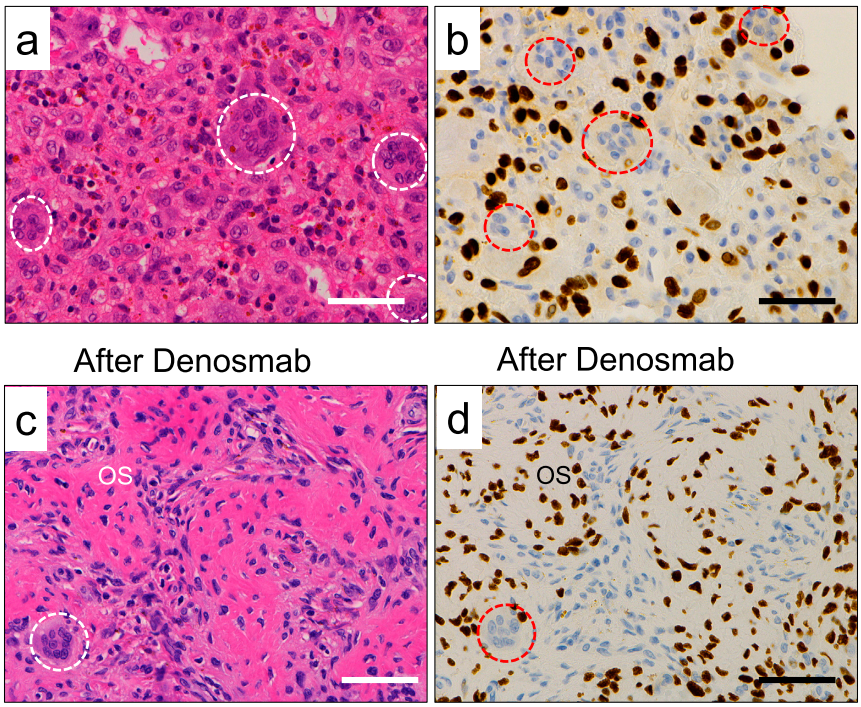

Fig. 5. Histological analysis of giant cell tumor of bone (GCTB) tissue sections using anti-H3.3 G34W antibody. (**a**, HE stain) Biopsied specimen of giant cell tumor of bone (GCTB). Numerous osteoclast-type multinucleated giant cells are observed in the background of mononuclear cell proliferation. Circles indicate multinucleated giant cells. (**b**) Immunohistochemical staining with anti-H3.3 G34W antibody. Nuclei of spindle-shaped cells are positive for H3.3 G34W, but giant cells are negative. (**c**, HE stain) Resected specimens after Denosmab administration to GCTB. Osteoclast-type giant cells are markedly decreased, and osteoid (OS) formation has become prominent. (**d**) Immunohistochemical staining with anti-H3.3 G34W antibody. Nuclei of spindle-shaped cells and osteoblasts are H3.3 G34W positive. Bars = 100 μm.

Chondroblastoma: with H3K36M antibody

Chondroblastoma is a benign tumor that develops in cartilage at the end of bone in adolescents and young adults. Mononuclear chondroblasts proliferate solidly with osteoclast-like giant cells, have a cartilaginous matrix with calcification, and show various degrees of cartilage differentiation. Since approximately 90% of chondroblastoma cases harbor H3K36M by a mutation from lysine 36 (K36) to methionine in the H3F3B gene that encodes H3.3 [3], immunostaining with an antibody that recognizes H3K36M is effective in confirming the histopathological diagnosis of chondroblastoma.

IV. Conclusion

Post-translational histone modifications play a direct role in changing the chromatin structure, while being involved in transcriptional regulation through chromatin remodeling complexes, acetylase/deacetylase, methylase/demethylase, and transcriptional coactivators. Recent studies on cancer genomics have indicated that many driver mutations that result in carcinogenesis are located in genes encoding chromatin modifiers. The detailed mechanisms of cellular oncogenesis caused by histone mutations will be elucidated in future studies. Chromatin dynamics, being the interface between cancer genome knowledge and epigenomic regulatory mechanisms, is directly related to routine histopathological diagnosis. We believe this review will help make histone modification and chromatin remodeling more familiar and accessible under microscopic observation.

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VI. Conflicts of Interest

The authors have no conflicts of interest.

VII. References

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