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Epigenetic alterations in bone and soft tissue tumors

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Abstract

Human malignancies are driven by heritable alterations that lead to unchecked cellular proliferation, invasive growth and distant spread. Heritable changes can arise from changes in DNA sequence, or, alternatively, through altered gene expression rooted in epigenetic mechanisms. In recent years, high-throughput sequencing of tumor genomes has revealed a central role for mutations in epigenetic regulatory complexes in oncogenic processes.^{1–3} Through interactions with or direct modifications of chromatin, these proteins help control the accessibility of genes, and thus the transcriptional profile of a cell. Dysfunction in these proteins can lead to activation of oncogenic pathways or silencing of tumor suppressors.

While epigenetic regulators are altered across a broad spectrum of human malignancies, they play a particularly central role in tumors of mesenchymal and neuroectodermal origin (table 1). This review will focus on recent advances in the understanding of the molecular pathogenesis of a subset of tumors in which alterations in the polycomb family of chromatin modifying complexes, the SWI/SNF family of nucleosome remodelers, and histones play a central role in disease pathogenesis. While this review will focus predominantly on the molecular mechanisms underlying these tumors, each section will also highlight areas in which an understanding of the molecular pathogenesis of these diseases has led to the adoption of novel immunohistochemical and molecular markers.

Introduction

The basic subunit of chromatin is the nucleosome. Each nucleosome consists of 146 bp of DNA wound around an octameric assembly of histone proteins consisting of two copies each of histone H2A, H2B, H3 and H4 (Figure 1A). Nucleosomes are in turn packaged into higher-order assemblies including chromatin fibers, domains or, for the purposes of mitosis, chromosomes.⁴ The accessibility of genes for transcription is influenced by the higher-order structure of chromatin, the density of nucleosome packing and a variety of protein and nucleic acid factors that bind to and interact with chromatin.

One group of chromatin modifiers influences gene accessibility through enzymatic modification of histone protein tails (1B, right, arrows). Histone tails may be decorated with a wide array of post-translational modifications that influence the structure of the

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nucleosome and the binding of accessory factors that can increase or decrease the likelihood that a gene is transcribed.^{5,6} Some of these modifications have been characterized as associated with either a relatively loose, open chromatin state that is permissible to transcription (green dots) or a compacted, repressed state (red dots) in which the gene is not transcribed.

The SWI/SNF family of nucleosome remodelers and the polycomb family of transcriptional repressors are among the best characterized epigenetic regulatory complexes, and are associated with active and repressed chromatin, respectively. The SWI/SNF family consists of a number of tissue and cell-type specific multi-protein complexes that alter chromatin structure in an ATP-dependent manner.^{7,8} They remove nucleosomes and enhance the deposition of active histone modifications, thereby promoting an open, transcriptionally competent state. Polycomb complexes—including polycomb repressive complex 1 (PRC1), polycomb repressive complex 2 (PRC2) and related complexes—have the opposite effect. Following recruitment to DNA, polycomb complexes deposit histone modifications associated with transcriptionally silent chromatin, and promote chromatin compaction.^{9–11}

Opposition between these protein families was first described in *Drosophila* development where mutations in *brhma* (a *Drosophila* homolog of a SWI/SNF member) suppressed polycomb-mutant phenotypes.¹² Since that time, extensive work has characterized the opposition of these complexes in developmental processes.^{9–11,13} This work highlights the fact that basic cellular function requires a balance between the activities of complexes promoting gene expression and those promoting gene silencing.

Mutations in the genes encoding members of chromatin altering complexes are among the commonest alterations in human malignancies, and they disrupt the balance between active and repressed chromatin.¹⁴ These mutations can function as oncogenes or tumor suppressors, depending on the cellular context and the identities of the genes inappropriately expressed or silenced. Tumors may depend on continued epigenetic imbalance for unchecked proliferation, and restoration of the balance can halt tumor growth. For example, SWI/SNF-mutant cancers are particularly sensitive to loss of PRC2 components.^{15,16} Each section below will address alterations in particular components of an epigenetic complex and, when known, how they result in tumor formation. We will also briefly mention immunohistochemical or molecular markers associated with the relevant alterations in each of these tumors.

SMARCB1 mutant sarcomas

Several human sarcomas are characterized by recurrent losses of the SWI/SNF family member SMARCB1 (also known as *Ini1* and *hSNF5*—see table 1). The first of these to be characterized was malignant rhabdoid tumor, a highly aggressive malignancy that often occurs in the kidney of young children, but may arise at a variety of extrarenal sites and within the central nervous system.¹⁷ It is characterized by enlarged, polygonal cells with eccentric, eosinophilic cytoplasm, vesicular chromatin and a prominent nucleolus. Over two decades ago, malignant rhabdoid tumor became the first characterized malignancy in which loss-of-function mutation of a SWI/SNF family member was shown to be the primary

driver.¹⁸ In malignant rhabdoid tumor, biallelic loss of SMARCB1 occurs on a nearly normal genetic background, indicating that it can function as a potent oncogenic driver.¹⁹

A number of studies have elucidated the mechanism by which SMARCB1 loss leads to cellular transformation. It was initially shown that malignant rhabdoid tumor was driven by inactivation of the CDKN2A tumor suppressor (P16INK4A).²⁰ Subsequent studies revealed that CDKN2A inactivation was driven by polycomb-mediated epigenetic silencing of this locus.¹⁵ Most recently, this has been shown to be due, at least in part, to direct physical opposition of polycomb and SWI/SNF complexes on chromatin. Using a cleverly engineered system, researchers established that recruitment of SWI/SNF complexes to polycomb-silenced chromatin led to polycomb eviction and loss of histone modifications associated with transcriptional repression.^{21,22} SWI/SNF complexes lacking SMARCB1 could not evict polycomb, leaving repressive modifications in place. This work provided a physical mechanism for the general model that SWI/SNF loss leads to unopposed polycomb activity and concomitant silencing of critical tumor suppressor genes. Indeed, this general model is thought to play a role in other malignancies in which SMARCB1 or other SWI/SNF members are lost. The growing list of such tumors includes several sarcomas, a subset of undifferentiated or dedifferentiated carcinomas, and tumors of unclear histogenesis (undifferentiated thoracic sarcomas and small cell carcinoma of the ovary, hypercalcemic type).^{17,23–26}

Diagnostic utility of SMARCB1(Ini1) loss in mesenchymal tumors

The finding of SMARCB1 loss in malignant rhabdoid tumor led to its adoption as a diagnostic marker.²⁷ SMARCB1 loss remains a sensitive and specific marker for malignant rhabdoid tumor in the appropriate clinical and histologic context. It has also been adopted as a sensitive and specific marker for epithelioid sarcoma, both proximal and distal types (Figure 2A), the overwhelming majority of which have loss of Ini1 expression, most commonly associated with biallelic deletions in the SMARCB1 gene.²⁸ Proximal and distal epithelioid sarcomas share a name and some overlapping morphologic and immunohistochemical features. Both of these tumors may exhibit loosely cohesive round to epithelioid cells. Both share expression of keratin, CD34 and loss of Ini1. Despite these similarities, there are some clinical and morphologic differences. The distal type most commonly presents in the superficial soft tissues of the extremities, often with ulceration of the overlying skin. The distal type is also more likely to have the classic ‘granuloma-like’ appearance, with a small central area of necrosis with surrounding epithelioid tumor cells resembling histiocytes. Those categorized as ‘proximal’ type epithelioid sarcoma, present at proximal and deep soft tissue locations, often as a large mass. They tend to contain larger cells with greater cytologic atypia and more pronounced mitotic activity. They also have a greater propensity for sheet-like growth.²⁹ Proximal-type epithelioid sarcomas behave in a somewhat more aggressive fashion, but there is some evidence that they may respond better to cytotoxic chemotherapy.³⁰

Since epithelioid sarcoma exhibits strong keratin reactivity, it may be mistaken for a poorly differentiated carcinoma. Moreover, loss of Ini1 staining can be seen in poorly-differentiated carcinomas, either through alterations in SMARCB1 or other SWI/SNF components, further

complicating this distinction. Recently, the tight-junction protein claudin-4 has been proposed as a specific marker for the distinction between poorly differentiated carcinoma with *Ini1* loss and epithelioid sarcoma. In a series of 60 sarcomas with *Ini1* loss, claudin-4 expression was seen in only 3 tumors (2 myoepithelial carcinomas and 1 malignant rhabdoid tumor). Conversely, claudin-4 was expressed in 16/20 poorly-differentiated carcinomas.³¹

Over the years, loss of *SMARCB1* expression has also been demonstrated in a variety of tumors including several other sarcomas. These include subsets of epithelioid MPNST and soft tissue myoepitheliomas and at least rare cases of extraskeletal myxoid chondrosarcomas.^{17,26} In the case of epithelioid MPNST, it is present in a sufficiently large number (~50%) that it may aid in the diagnosis in the proper histologic setting with the support of other markers. In myoepithelioma and extraskeletal myxoid chondrosarcoma, it is a less common finding, but one to be aware of to avoid misinterpretation.

Mutations in PRC2 in Malignant Peripheral Nerve Sheath Tumor (MPNST)

MPNST is an aggressive sarcoma originating from supporting cells of peripheral nerves. MPNSTs may arise spontaneously, in the context of neurofibromatosis type I, or following radiation therapy. Early genetic studies revealed complex alterations, with numerous mutations, gains and losses, but few recurrent mutations outside of *NF1* and *p53*. Recently, however, two groups published simultaneous reports of recurrent alterations in members of the polycomb repressive complex 2 (PRC2) in MPNST.^{32,33} The mutations were most commonly found in the subunit *SUZ12*, with a smaller subset harboring mutations in the *EED* subunit. In some instances, *SUZ12* loss was the result of germ-line co-deletion in *NF1* patients, since *SUZ12* neighbors *NF1* on chromosome 17. Both *SUZ12* and *EED* are core components of the PRC2 complex, which promotes transcriptional silencing by catalyzing the deposition of the repressive trimethyl modification on lysine 27 of the tail of histone H3 (H3K27me3). In both papers, the authors noted global loss of H3K27me3 in PRC2 mutant MPNST.

A comparison of gene expression profiles in PRC2 mutant and PRC2 retained MPNST revealed that, consistent with its role as a transcriptional repressor, loss of PRC2 predominantly led to gene activation.³³ Among the activated genes were well-characterized targets of PRC2 repression, including developmental programming genes such as those in the *Hox* family. Thus, loss of PRC2 was associated with the inappropriate activation of genes expressed in earlier progenitors. Subsequent work on cell culture models of MPNST showed that PRC2 loss also potentiated activation of the Ras pathway.³⁴ This pathway, already active due to loss of *NF1*, a Ras-GTPase activating protein, was further amplified upon PRC2 loss. Ras pathway amplification was associated with changes in histone post-translational modifications, most notably a switch from methylation to acetylation at H3K27 (H3K27me3→H3K27ac). This latter modification is associated with enhancers, which strongly promote transcription of target genes. The authors also found that PRC2 loss led to tumor susceptibility to BRD4 inhibitors, which directly interfere with the ability of activating chromatin modifications to promote transcription.

The model of MPNST pathogenesis derived from these studies is essentially the opposite of that described for malignant rhabdoid tumor. Rather than unopposed polycomb activity and transcriptional silencing of tumor suppressors, there is a loss of silencing, leading to unopposed activation of genes amplifying signaling pathways involved in growth and division.

The finding of global H3K27me3 loss in MPNST has been adopted as a novel immunohistochemical marker (Figure 2B). At least four large studies have reported on the specificity and sensitivity of H3K27me3 loss in the diagnosis of MPNST.^{35–38} Most studies have found good specificity, with rare examples of H3K27me3 loss in other sarcomas. However, one large series noted H3K27me3 loss in a subsets of synovial sarcomas and fibrosarcomatous dermatofibrosarcoma protuberans (9/15 and 3/8, respectively), two close histologic mimics of MPNST.³⁵ The reported sensitivity values of H3K27me3 loss have been both more variable and less impressive (34–61%). In addition to its utility as a diagnostic marker, several series also found loss of H3K27me3 to have prognostic significance, as it was associated with aggressive disease.

Synovial Sarcoma: Driven by translocation of a SWI/SNF family member

Synovial sarcoma a spindle-cell sarcoma most commonly presenting in the deep soft tissues of the extremities, but also occurring at a wide array of anatomic sites. It has a broad age distribution, but typically presents in young patients with both mean and median ages of presentation in the early to mid-30s.³⁹ Synovial sarcoma contains a fusion of the SYT gene (also known as SS18) on chromosome 18 with either the SSX1 or SSX2 gene on the X chromosome.

Both gene products involved in SYT-SSX fusions associate with epigenetic regulatory complexes: SYT with the SWI/SNF complex and SSX with polycomb.^{40,41} Early on, researchers focused on the idea of polycomb gain-of-function and tumor suppressor silencing as the key to pathogenesis. This work established that polycomb silenced the tumor suppressor gene early growth response 1 (EGR1) in an SS18-SSX dependent fashion and that this silencing could be reversed by histone deacetylase inhibitors, which promote the restoration of active chromatin.⁴² This model was later expanded when it was discovered that SYT-SSX targeted specific tumor suppressor genes for silencing through an association with transducing-like enhancer of split-1 (TLE-1) and activating transcription factor 2 (ATF2).⁴³ This latter study characterized the role of TLE-1, a commonly employed immunohistochemical marker of synovial sarcoma, in disease pathogenesis. Together, these studies highlighted a role for polycomb and gene silencing in synovial sarcoma pathogenesis. The recent finding that pre-clinical models of synovial sarcoma are sensitive to inhibition of EZH2, the enzymatic component of the polycomb PRC2 complex, is also consistent with a role for polycomb in synovial sarcoma pathogenesis.⁴⁴

A separate set of experiments suggests a different model, in which SWI/SNF plays the lead role. Kadoch et al demonstrated that SS18 was an integral component of the SWI/SNF complex, and that the SS18-SSX fusion protein dominantly assembles into SWI/SNF complex, leading to loss of the SMARCB1 subunit.^{21,40} This fusion-containing SWI/SNF

complex potently evicts polycomb, leading to loss of PRC2 components and their associated repressive histone modifications along broad regions of DNA. This expands the region of chromatin permissible for transcription, leading to transcriptional activation of oncogenes, most notably Sox2, driving tumorigenesis. This model also provides an explanation for prior histologic studies, which show reduced levels of SMARCB1/Ini1 staining in synovial sarcoma.⁴⁵

It is possible that these different models are the result of distinct experimental methods or conditions, and that one or the other will prove to be the dominant mechanism for transformation. Indeed, preliminary clinical trial data showed no objective responses to the EZH2 inhibitor tazemetostat in patients with advanced synovial sarcomas, suggesting that a hyperactive polycomb complex is not likely central to disease pathogenesis.⁴⁶ Nonetheless, since the trial was conducted in a select group with advanced disease, it remains possible that both mechanisms contribute at some level. One way to reconcile these seemingly discordant findings is that, rather than completely eliminating polycomb, the gain-of-function phenotype effects a redistribution such that SWI/SNF binds and evicts polycomb from SWI/SNF target genes, de-repressing critical drivers of tumorigenesis, including Sox2. Meanwhile, evicted polycomb reassembles at other sites leading to localized areas of increased activity and transcriptional silencing of critical tumor suppressors, including EGR1. In this model, pathogenesis is driven by a reshuffling of chromatin structure which results in the activation of some genes and repression others. This mechanism is more complex than global loss of function phenotypes in SWI/SNF and polycomb described for malignant rhabdoid tumor and MPNST, respectively. It is similar to that recently described for a histone H3.3 H3K27M mutation in pediatric gliomas. These tumors exhibit extensive loss of H3K27me3 across the genome yet also have focal areas of increased polycomb activity, including at the critical tumor suppressor CDKN2A.^{47–49}

Recurrent polycomb-member rearrangements characterize a diverse set of sarcomas

Several histologically and clinically distinct soft tissue tumors/sarcomas harbor rearrangements in members of the extended polycomb family. These include endometrial stromal sarcoma, ossifying fibromyxoid tumor and the relatively recently characterized group of sarcomas containing BCOR alterations. While these rearrangements already serve as the basis for specific molecular diagnostic tests—either by fluorescence *in-situ* hybridization (FISH) or next-generation sequencing, less is known about the way in which translocations promote tumorigenesis.

Endometrial stromal nodule and low-grade endometrial sarcoma

Endometrial stromal nodule is a circumscribed proliferation of monomorphic ovoid uterine mesenchymal cells. Its malignant counterpart, low-grade endometrial stromal sarcoma, exhibits infiltrative growth of the surrounding myometrium.

Both endometrial stromal nodule and low-grade endometrial stromal sarcoma harbor recurrent translocations involving polycomb family members.^{50–53} The first characterized

and most common of these fusions is a t(7;17)(p15;q21) translocation which fuses JAZF1, a zinc-finger DNA binding protein, to SUZ12, a core member of PRC2.^{54,55} Interestingly, the mRNA encoding the fusion gene was shown to naturally occur in endometrial stromal cells as a consequence of trans-splicing of mRNA, suggesting that oncogenesis is driven by constitutive expression of a fusion protein that is otherwise expressed in a regulated fashion.⁵⁶ Numerous other fusions have been characterized, each of which involves the joining of a polycomb member to another protein involved, directly or indirectly, with DNA interactions.⁵¹ Tumors with any of the fusions share the same hallmark morphologic features, and none has been shown to have distinct prognostic value within the category of low-grade endometrial stromal sarcoma.

There have been relatively few studies looking at the function of fusion proteins in endometrial stromal sarcoma, and all of them have focused on the JAZF1-SUZ12 fusion. As noted above, the fusion is found both in endometrial stromal nodule and endometrial stromal sarcoma. Progression from endometrial stromal nodule to endometrial stromal sarcoma was shown to correlate with loss of expression of the remaining normal SUZ12 allele. In a tissue culture system, the fusion protein was capable of promoting increased growth, but only when the normal SUZ12 allele was suppressed with RNAi knock-down.⁵⁷ More recent work demonstrated that the fusion protein destabilizes the PRC2 complex *in vitro* and in tissue culture, reducing both methyltransferase activity and PRC2 localization to several known polycomb target genes. This led to a model in which gene fusions lead to PRC2 loss-of-function and presumed activation of growth-promoting genes.⁵⁸

This model is similar to that proposed for MPNST, and suggests a straightforward loss-of-function. Intuitively, it seems unlikely that recurrent rearrangements involving specific protein members of related complexes simply result in loss of function. Moreover, these studies were not carried out on endometrial stromal sarcoma cell lines, with endogenous fusion expression. Thus, additional work will be required to validate these results, or to determine whether more complex mechanisms are at play, such as altered activity or targeting on the fusion protein.

Ossifying fibromyxoid tumor

Ossifying fibromyxoid tumor is a tumor of uncertain histogenesis that typically occurs in the superficial soft tissues of the extremities. The name is derived from the characteristic shell of lamellar bone found at the periphery of the tumor in more than half of cases.²⁹ It is generally cured with local excision, but atypical and malignant forms may exhibit more aggressive behavior. Early cytogenetic studies identified recurrent rearrangements involving the PHF1 locus on chromosome 6.^{59,60} The fusion landscape of ossifying fibromyxoid tumor has been more thoroughly defined in two recent papers. In the combined series, researchers analyzed over 40 cases and found multiple different fusions.^{61,62} The PHF1 gene was the C-terminal partner in the fusion protein in the majority of cases (~80%), with EP400 as the most common N-terminal partner (44%). Other PHF1 fusion partners included EPC1 and MEAF6. In addition to these rearrangements, the investigators found a number of other fusions containing proteins involved in epigenetic pathways, including BCOR (ZC3H7B-BCOR), BCORL1 (CREBBP-BCORL1) and KDM2A-WWTR1.⁶² All of these fusions have

two components: one partner that engages with proteins or complexes associated with active chromatin and another involved in transcriptional silencing, usually through association with members of the polycomb family. Of note, several of the fusions are seen in both ossifying fibromyxoid tumor and endometrial stromal sarcoma, and one (ZC3H7B-BCOR) is seen in primitive round cell sarcomas, suggesting shared mechanisms of molecular pathogenesis among these tumors. As with endometrial stromal sarcoma, the molecular mechanisms for these fusions have not been elucidated, nor is it clear whether all of the diverse fusions have the same effects on histone post-translational modifications and gene expression. Nonetheless, given the functional roles of the proteins involved, it seems highly likely that the fusion proteins alter the distribution of histone modifications thereby promoting active or silent chromatin states, either locally or globally. In line with what we have seen in the better characterized cases above, this likely leads to activation of one or more oncogenes and repression of one or more tumor suppressors.

BCOR-altered sarcomas

BCOR (B-cell lymphoma 6 corepressor) is a member of the polycomb family and functions as a transcriptional corepressor through its involvement in a non-canonical PRC1 complex.⁶³ The BCOR complex plays a significant role in mesenchymal differentiation by promoting the removal of activating histone post-translational modifications and the deposition of repressive post-translational modifications, leading to transcriptional silencing. Mutations in BCOR occur in a rare genetic syndrome called oculofaciocardiodental syndrome (OFCD), named for a constellation of abnormalities in the eye, face, heart and teeth. These abnormalities are thought to be a result of aberrant transcription resulting from an increase in activating H3K4 and H3K36 methylation modifications due to BCOR loss of function in mesenchymal progenitor cells.⁶⁴

Recent genomic and transcriptomic analyses have identified recurrent alterations in the BCOR gene in several distinct mesenchymal malignancies. A paracentric inversion of the X-chromosome resulting in a fusion protein merging BCOR with CCNB3, a testis specific cyclin, was first reported in a series of undifferentiated round cell sarcomas of bone.⁶⁵ These BCOR-CCNB3 fusion sarcomas occur in children and adolescents, and show a substantial male predominance. Since the identification of BCOR-CCNB3 fusions, other BCOR fusions have been reported in undifferentiated round cell sarcomas occurring in older, predominantly male patients, including BCOR-MAML3 and ZC3H7B-BCOR fusions.⁶⁶

In addition to these fusions, BCOR internal tandem duplications have been identified in several histomorphologically distinct mesenchymal malignancies including undifferentiated small round blue cell sarcomas of infants, as well as in clear cell sarcoma of the kidney and primitive myxoid mesenchymal tumor of infancy.^{67–69}

While originally identified in small round blue cell tumors, tumors with BCOR-alterations are morphologically diverse. The tumors described above range from those having small, primitive round cells, to those containing a spindled appearance with variable amounts of myxoid stroma. This is true both across all of the tumor types, and even within subsets harboring the same rearrangement. For example, BCOR internal tandem duplications are seen in the spindled clear cell sarcoma of the kidney, undifferentiated round cell sarcomas,

and the variable-appearing spindled, round and myxoid primitive myxoid mesenchymal tumor of infancy. Similarly, the morphologic spectrum of BCOR-CCNB3 fusion sarcomas is broad, containing round, spindled, myxoid and, in rare cases, even pleomorphic tumors, though this latter morphology may be due in part to therapy.^{70–73}

The morphologic diversity of these tumors presents a diagnostic challenge. Thankfully, biomarkers linked directly to the fusion have proved valuable diagnostic tools. CCNB3 IHC is both specific and sensitive for the detection of BCOR-CCNB3 fusion sarcomas. BCOR IHC, while less specific, is a sensitive marker for tumors with BCOR alterations, including those with internal tandem duplications and variant rearrangements.⁶⁷ BCOR alterations may also be detected by reverse-transcription polymerase chain reaction (rtPCR) and FISH using customized probes. The molecular pathophysiology of these rearrangements is not well characterized. In the case of BCOR-CCNB3 sarcomas, researchers have demonstrated that overexpression of just the CCNB3 portion of the fusion gene can promote cell division to a similar extent as the fusion protein. This suggests that, at least with respect to cell-cycle progression, CCNB3 plays a significant role.⁶⁵ However, the identification of non-CCNB3 BCOR fusion partners, and tumors harboring only BCOR internal tandem duplications, suggests that BCOR may be the dominant driver. In support of this, transcriptional profiling of some BCOR-rearranged sarcomas suggests inappropriate activation of Hox genes, which are targets of polycomb-mediated repression. While these findings are intriguing, it is unclear if or how BCOR drives transcriptional changes in these tumors, and if alterations in histone post-translational modifications play a major role.

Mutations in the histones themselves: Giant cell tumor of bone and Chondroblastoma

To this point, each tumor discussed has had recurrent alterations in proteins responsible for modifying gene accessibility through effects on histone proteins. Mutations in the histones themselves may also alter the structure of chromatin. Recent high-throughput sequencing studies have identified specific recurrent mutations in histone proteins in a number of human neoplasms.⁷⁴ The consequences of these mutations have been studied in several cases, and they appear to globally alter the deposition of histone post-translational modifications, leading to changes in gene expression. For example, diffuse intrinsic pontine glioma is characterized by recurrent H3K27M mutations in H3.3, a histone H3 variant. This mutation leads to a marked global loss of the silencing modification H3K27me3.⁷⁵ Interestingly, it also led to accumulation of the limited remaining H3K27me3 at certain genes.^{47–49} The effect of this mutation is therefore activation of many genes and silencing of a few key others. Thus, histone mutations can greatly alter chromatin accessibility and gene expression, but the effects of individual mutations are not necessarily straightforward.

In the past several years, histone mutations have been identified in two different mesenchymal lesions of bone: chondroblastoma and giant cell tumor of bone. Both have recurrent driver mutations in histone H3.3, a replication-independent histone H3 variant, that is incorporated at sites of active transcription.⁷⁶ H3.3 is encoded by two genes, H3F3A and H3F3B, and the mutations identified to date have been almost entirely subtype specific, with

chondroblastoma having mutations in the H3F3B gene, and giant cell tumor of bone having mutations in the H3F3A gene.⁷⁷ The presence of specific recurrent mutations has led to the development of novel diagnostic tests either in the form of molecular analysis of the DNA sequence,^{78,79} or immunohistochemistry using mutation-specific antibodies (Figure 2 C&D).^{80,81} These novel diagnostic tests are likely to be particularly useful on needle core biopsy specimens, or in cases with atypical clinical presentations. Immunohistochemistry in particular, which may be employed in most histologic labs, is highly promising (Figure 2C and 2D). Mutant-specific antibodies—for H3K36M mutant protein in chondroblastoma and H3G34W mutation in giant cell tumor—have shown both excellent sensitivity and specificity.^{80,81}

Chondroblastoma

Chondroblastoma is a benign primary bone neoplasm that typically occurs in the epiphysis of long bones in skeletally immature persons.⁸² Histologically, it is characterized by an ovoid mononuclear population with nuclear grooves, a moderate amount of eosinophilic cytoplasm and well-defined cell borders (Figure 2C). In some areas, the matrix mineralizes around individual cells leading to the hallmark chicken-wire pattern of calcification. These are accompanied by numerous osteoclast-type giant cells and patchy areas of immature cartilaginous matrix. Standard treatment is by curettage, with relatively low recurrence rates.

A recurrent K36M mutation in H3F3B, a gene encoding the histone H3 variant H3.3, was recently found in the vast majority of chondroblastomas.⁷⁷ Like the H3K27M mutation in gliomas discussed above, this mutation substitutes a methionine residue for a lysine residue at a site that is subject to post translational modification. Methylation at H3K36 is associated with active transcription.⁸³ In cell culture models, H3K36M mutation dramatically decreased global H3K36 methylation levels and showed a concomitant increase in global H3K27me3 levels.⁸⁴ This was associated with impaired differentiation of several different cell types. Of note, the increased levels of H3K27me3 did not lead to global transcriptional repression. Rather, there was a mixed effect, with most genes activated but others repressed. The authors attributed this to a shift in the distribution of H3K27me3, from gene-associated to intergenic. The intragenic H3K27me3 functioned as a sink, recruiting other proteins involved in silencing away from promoter-associated H3K27me3 peaks. In this model, while there was a global increase in H3K27me3, there was a relative decrease in the gene-associated peaks responsible for transcriptional repression (Figure 3).

Giant cell tumor of bone

Giant cell tumor of bone is a locally-aggressive tumor occurring predominantly in the metaphysis of long bones in skeletally mature patients. It is a giant-cell rich lesion, characterized by numerous, evenly-distributed massive osteoclast-type giant cells within a background of ovoid mononuclear cells (Figure 2D). These latter cells are the neoplastic component, and induce the proliferation of the eponymous giant-cells through high-level expression of receptor activator of nuclear factor kappa-B ligand (RANKL).⁸⁵

As with chondroblastoma, giant cell tumor contains recurrent driver mutations in a gene encoding histone H3.3. However, whereas chondroblastoma mutations are typically in the H3F3B gene, giant cell tumor mutations are overwhelmingly in H3F3A, with only rare cases of H3F3B mutations. Over 90% of these tumors harbor mutations in H3F3A which result in substitutions at G34. The vast majority harbor G34W substitutions (>95% of total mutations identified), but other substitutions, including G34V, G34R, G34L and S28N have been found in rare cases.^{77,79,80} Nearly all of the alterations affect the G34 position. While this residue itself is not involved in histone post-translational modifications, it is adjacent to H3K36, which can be post-translationally modified by methylation or acetylation as discussed above. The H3F3A mutations in giant cell tumor may affect histone post-translational modification deposition through a steric effect, given the close proximity to H3K36. It is also possible that these mutations affect post-translational modifications at more distant sites. Further work will be required to assess the extent to which these mutations alter global histone post-translational modifications, and how and whether such changes influence transcription of key tumor-mediating genes, as has been seen in other contexts.⁸⁶

Concluding remarks

Genes encoding proteins involved in epigenetic regulation through chromatin modification are frequently mutated or lost in human cancers including many tumors of bone and soft tissue. Alterations in epigenetic regulators promote tumor formation by altering the expression of numerous target genes. In several of the malignancies described above, some of the critical downstream targets of altered epigenetic regulation are known (e.g. CDKN2A in malignant rhabdoid tumor and epithelioid sarcoma, Sox2 in synovial sarcoma). In others, the targets remain to be characterized. While this review has stressed antagonism between polycomb and SWI/SNF, numerous other chromatin modifiers are undoubtedly involved in tumorigenesis. Intensive investigations are underway to characterize the way in which alterations in epigenetic regulators affect gene accessibility and expression, and how and why these changes result in tumor progression. These will undoubtedly lead to novel therapies as well as new prognostic and diagnostic markers, some directly reflecting changes in histone modifications (as in H3K27me3 detection in MPNST) and others reflecting genes uniquely activated by epigenetic mechanisms.

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References

1. Dawson MA, Kouzarides T. Cancer Epigenetics: From Mechanism to Therapy. *Cell*. 2012; 150:12–27. [PubMed: 22770212]

2. Gnad F, Doll S, Manning G, Arnott D, Zhang Z. Bioinformatics analysis of thousands of TCGA tumors to determine the involvement of epigenetic regulators in human cancer. *BMC Genomics*. 2015; 16(Suppl 8):S5.
3. Huether R, et al. The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. *Nat Commun*. 2014; 5:3630. [PubMed: 24710217]
4. Woodcock CL, Ghosh RP. Chromatin higher-order structure and dynamics. *Cold Spring Harb Perspect Biol*. 2010; 2:a000596–a000596. [PubMed: 20452954]
5. Huang H, Sabari BR, Garcia BA, Allis CD, Zhao Y. SnapShot: histone modifications. *Cell*. 2014; 159:458–458e1. [PubMed: 25303536]
6. Zhao Y, Garcia BA. Comprehensive Catalog of Currently Documented Histone Modifications. *Cold Spring Harb Perspect Biol*. 2015; 7:a025064. [PubMed: 26330523]
7. Kadoch C, Crabtree GR. Mammalian SWI/SNF chromatin remodeling complexes and cancer: Mechanistic insights gained from human genomics. 2015; 1:e1500447–e1500447.
8. Kadoch C, Copeland RA, Keilhack H. PRC2 and SWI/SNF Chromatin Remodeling Complexes in Health and Disease. *Biochemistry*. 2016; acs.biochem.5b01191-15. doi: 10.1021/acs.biochem.5b01191
9. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature*. 2011; 469:343–349. [PubMed: 21248841]
10. Piunti A, Shilatifard A. Epigenetic balance of gene expression by Polycomb and COMPASS families. *Science*. 2016; 352:aad9780–aad9780. [PubMed: 27257261]
11. Aranda S, Mas G, Di Croce L. Regulation of gene transcription by Polycomb proteins. *Science Advances*. 2015; 1:e1500737–e1500737. [PubMed: 26665172]
12. Tamkun JW, et al. brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell*. 1992; 68:561–572. [PubMed: 1346755]
13. Kennison JA. The Polycomb and trithorax group proteins of Drosophila: trans-regulators of homeotic gene function. *Annu Rev Genet*. 1995; 29:289–303. [PubMed: 8825476]
14. Kadoch C, et al. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. *Nat Genet*. 2013; 45:592–601. [PubMed: 23644491]
15. Wilson BG, et al. Epigenetic Antagonism between Polycomb and SWI/SNF Complexes during Oncogenic Transformation. *Cancer Cell*. 2010; 18:316–328. [PubMed: 20951942]
16. Kim KH, et al. SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. *Nature Medicine*. 2015; 21:1491–1496.
17. Hollmann TJ, Hornick JL. INI1-Deficient Tumors. *Am J Surg Pathol*. 2011; 35:e47–e63. [PubMed: 21934399]
18. Versteeg I, et al. Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. *Nature*. 1998; 394:203–206. [PubMed: 9671307]
19. Lee RS, et al. A remarkably simple genome underlies highly malignant pediatric rhabdoid cancers. *J Clin Invest*. 2012; 122:2983–2988. [PubMed: 22797305]
20. Oruetxebarria I. p16INK4a Is Required for hSNF5 Chromatin Remodeler-induced Cellular Senescence in Malignant Rhabdoid Tumor Cells. *Journal of Biological Chemistry*. 2003; 279:3807–3816. [PubMed: 14604992]
21. Kadoch C, et al. Dynamics of BAF–Polycomb complex opposition on heterochromatin in normal and oncogenic states. *Nat Genet*. 2016; 49:213–222. [PubMed: 27941796]
22. Stanton BZ, et al. Smarca4 ATPase mutations disrupt direct eviction of PRC1 from chromatin. *Nat Genet*. 2016; 49:282–288. [PubMed: 27941795]
23. Le Loarer F, et al. SMARCA4 inactivation defines a group of undifferentiated thoracic malignancies transcriptionally related to BAF-deficient sarcomas. *Nat Genet*. 2015; 47:1200–1205. [PubMed: 26343384]
24. Jelinic P, et al. Recurrent SMARCA4 mutations in small cell carcinoma of the ovary. *Nat Genet*. 2014; 46:424–426. [PubMed: 24658004]
25. Witkowski L, et al. Germline and somatic SMARCA4 mutations characterize small cell carcinoma of the ovary, hypercalcemic type. *Nat Genet*. 2014; 46:438–443. [PubMed: 24658002]

26. Agaimy A. The Expanding Family of SMARCB1(INI1)-deficient Neoplasia. *Advances In Anatomic Pathology*. 2014; 21:394–410. [PubMed: 25299309]
27. Hoot AC, Russo P, Judkins AR, Perlman EJ, Biegel JA. Immunohistochemical analysis of hSNF5/INI1 distinguishes renal and extra-renal malignant rhabdoid tumors from other pediatric soft tissue tumors. *Am J Surg Pathol*. 2004; 28:1485–1491. [PubMed: 15489652]
28. Sullivan LM, Folpe AL, Pawel BR, Judkins AR, Biegel JA. Epithelioid sarcoma is associated with a high percentage of SMARCB1 deletions. *Modern Pathology*. 2012; 26:385–392. [PubMed: 23060122]
29. Hoda SA. *Enzinger and Weiss's Soft Tissue Tumors*, 6th Edition. *Advances In Anatomic Pathology*. 2014; 21:216.
30. Kim C, et al. Different subtypes of epithelioid sarcoma and their clinical implication: long-term multi-institutional experience with a rare sarcoma. *APMIS*. 2017; 125:223–229. [PubMed: 28233450]
31. Schaefer IM, Agaimy A, Fletcher CD, Hornick JL. Claudin-4 expression distinguishes SWI/SNF complex-deficient undifferentiated carcinomas from sarcomas. *Modern Pathology*. 2017; doi: 10.1038/modpathol.2016.230
32. Zhang M, et al. Somatic mutations of SUZ12 in malignant peripheral nerve sheath tumors. *Nature Publishing Group*. 2014; 46:1170–1172.
33. Lee W, et al. PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. *Nature Publishing Group*. 2014; 46:1227–1232.
34. De Raedt T, et al. PRC2 loss amplifies Ras-driven transcription and confers sensitivity to BRD4-based therapies. *Nature*. 2014; 514:247–251. [PubMed: 25119042]
35. Cleven AHG, et al. Loss of H3K27 tri-methylation is a diagnostic marker for malignant peripheral nerve sheath tumors and an indicator for an inferior survival. *Mod Pathol*. 2016; 29:582–590. [PubMed: 26990975]
36. Prieto Granada CN, et al. Loss of H3K27me3 Expression Is a Highly Sensitive Marker for Sporadic and Radiation-induced MPNST. *Am J Surg Pathol*. 2016; 40:479–489. [PubMed: 26645727]
37. Schaefer IM, Fletcher CD, Hornick JL. Loss of H3K27 trimethylation distinguishes malignant peripheral nerve sheath tumors from histologic mimics. *Modern Pathology*. 2015; 29:4–13. [PubMed: 26585554]
38. Asano N, et al. Immunohistochemistry for trimethylated H3K27 in the diagnosis of malignant peripheral nerve sheath tumours. *Histopathology*. 2017; 70:385–393. [PubMed: 27580378]
39. Ladanyi M, et al. Impact of SYT-SSX fusion type on the clinical behavior of synovial sarcoma: a multi-institutional retrospective study of 243 patients. *Cancer Research*. 2002; 62:135–140. [PubMed: 11782370]
40. Kadoch C, Crabtree GR. Reversible Disruption of mSWI/SNF (BAF) Complexes by the SS18-SSX Oncogenic Fusion in Synovial Sarcoma. *Cell*. 2013; 153:71–85. [PubMed: 23540691]
41. Soulez M, Saurin AJ, Freemont PS, Knight JC. SSX and the synovial-sarcoma-specific chimaeric protein SYT-SSX co-localize with the human Polycomb group complex. *Oncogene*. 1999; 18:2739–2746. [PubMed: 10348348]
42. Lubieniecka JM, et al. Histone deacetylase inhibitors reverse SS18-SSX-mediated polycomb silencing of the tumor suppressor early growth response 1 in synovial sarcoma. *Cancer Research*. 2008; 68:4303–4310. [PubMed: 18519690]
43. Su L, et al. Deconstruction of the SS18-SSX Fusion Oncoprotein Complex: Insights into Disease Etiology and Therapeutics. *Cancer Cell*. 2012; 21:333–347. [PubMed: 22439931]
44. Shen JK, et al. Targeting EZH2-mediated methylation of H3K27 inhibits proliferation and migration of Synovial Sarcoma in vitro. *Sci Rep*. 2016; 6:25239. [PubMed: 27125524]
45. Kohashi K, et al. Reduced expression of SMARCB1/INI1 protein in synovial sarcoma. *Modern Pathology*. 2010; 23:981–990. [PubMed: 20305614]
46. Schoffski P, et al. Phase 2 multicenter study of the EZH2 inhibitor tazemetostat in adults with synovial sarcoma (NCT02601950). *Journal of Clinical Oncology*. 2017 Supplement–abstract 11057.

47. Chan KM, et al. The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev.* 2013; 27:985–990. [PubMed: 23603901]
48. Piunti A, et al. Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas. *Nature Medicine.* 2017; 23:493–500.
49. Mohammad F, et al. EZH2 is a potential therapeutic target for H3K27M-mutant pediatric gliomas. *Nature Medicine.* 2017; 23:483–492.
50. Chiang S, Oliva E. Cytogenetic and molecular aberrations in endometrial stromal tumors. *Hum Pathol.* 2011; 42:609–617. [PubMed: 21420714]
51. Chiang S, et al. Frequency of known gene rearrangements in endometrial stromal tumors. *Am J Surg Pathol.* 2011; 35:1364–1372. [PubMed: 21836477]
52. Allen AJ, Ali SM, Gowen K, Elvin JA, Pejovic T. A recurrent endometrial stromal sarcoma harbors the novel fusion JAZF1-BCORL1. *Gynecol Oncol Rep.* 2017; 20:51–53. [PubMed: 28331900]
53. Micci F, et al. MEAF6/PHF1 is a recurrent gene fusion in endometrial stromal sarcoma. *Cancer Letters.* 2014; 347:75–78. [PubMed: 24530230]
54. Nucci MR, Harburger D, Koontz J, Dal Cin P, Sklar J. Molecular analysis of the JAZF1-JJAZ1 gene fusion by RT-PCR and fluorescence in situ hybridization in endometrial stromal neoplasms. *Am J Surg Pathol.* 2007; 31:65–70. [PubMed: 17197920]
55. Koontz JI, et al. Frequent fusion of the JAZF1 and JJAZ1 genes in endometrial stromal tumors. *Proceedings of the National Academy of Sciences.* 2001; 98:6348–6353.
56. Li H, Wang J, Mor G, Sklar J. A neoplastic gene fusion mimics trans-splicing of RNAs in normal human cells. *Science.* 2008; 321:1357–1361. [PubMed: 18772439]
57. Li H, et al. Effects of rearrangement and allelic exclusion of JJAZ1/SUZ12 on cell proliferation and survival. *Proc Natl Acad Sci USA.* 2007; 104:20001–20006. [PubMed: 18077430]
58. Ma X, et al. The JAZF1-SUZ12 fusion protein disrupts PRC2 complexes and impairs chromatin repression during human endometrial stromal tumorigenesis. *Oncotarget.* 2017; 8:4062–4078. [PubMed: 27845897]
59. Gebre-Medhin S, et al. Recurrent rearrangement of the PHF1 gene in ossifying fibromyxoid tumors. *The American Journal of Pathology.* 2012; 181:1069–1077. [PubMed: 22796436]
60. Graham RP, et al. PHF1 rearrangements in ossifying fibromyxoid tumors of soft parts: A fluorescence in situ hybridization study of 41 cases with emphasis on the malignant variant. *Am J Surg Pathol.* 2013; 37:1751–1755. [PubMed: 23887158]
61. Antonescu CR, et al. Novel ZC3H7B-BCOR, MEAF6-PHF1, and EPC1-PHF1 fusions in ossifying fibromyxoid tumors-molecular characterization shows genetic overlap with endometrial stromal sarcoma. *Genes Chromosom Cancer.* 2013; 53:183–193. [PubMed: 24285434]
62. Kao YC, et al. Expanding the molecular signature of ossifying fibromyxoid tumors with two novel gene fusions: CREBBP-BCORL1 and KDM2A-WWTR1. *Genes Chromosom Cancer.* 2016; 56:42–50. [PubMed: 27537276]
63. Yamamoto Y, Abe A, Emi N. Clarifying the Impact of Polycomb Complex Component Disruption in Human Cancers. *Mol Cancer Res.* 2014; 12:479–484. [PubMed: 24515802]
64. Fan Z, et al. BCOR regulates mesenchymal stem cell function by epigenetic mechanisms. *Nat Cell Biol.* 2009; 11:1002–1009. [PubMed: 19578371]
65. Pierron G, et al. A new subtype of bone sarcoma defined by BCOR-CCNB3 gene fusion. *Nat Genet.* 2012; 44:461–466. [PubMed: 22387997]
66. Specht K, et al. Novel BCOR-MAML3 and ZC3H7B-BCOR Gene Fusions in Undifferentiated Small Blue Round Cell Sarcomas. *Am J Surg Pathol.* 2016; 40:433–442. [PubMed: 26752546]
67. Kao YC, et al. BCOR Overexpression Is a Highly Sensitive Marker in Round Cell Sarcomas With BCOR Genetic Abnormalities. *Am J Surg Pathol.* 2016; 40:1670–1678. [PubMed: 27428733]
68. Santiago T, Clay MR, Allen SJ, Orr BA. Recurrent BCOR internal tandem duplication and BCOR or BCL6 expression distinguish primitive myxoid mesenchymal tumor of infancy from congenital infantile fibrosarcoma. *Mod Pathol.* 2017; 6:40934.
69. Kenny C, et al. Mutually exclusive BCOR internal tandem duplications and YWHAE-NUTM2 fusions in clear cell sarcoma of kidney: not the full story. *J Pathol.* 2016; 238:617–620. [PubMed: 27000436]

70. Yamada Y, et al. Histological and immunohistochemical characteristics of undifferentiated small round cell sarcomas associated with CIC-DUX4 and BCOR-CCNB3 fusion genes. *Virchows Arch.* 2017; 470:373–380. [PubMed: 28197724]
71. Puls F, et al. BCOR-CCNB3 (Ewing-like) sarcoma: a clinicopathologic analysis of 10 cases, in comparison with conventional Ewing sarcoma. *Am J Surg Pathol.* 2014; 38:1307–1318. [PubMed: 24805859]
72. Peters TL, et al. BCOR-CCNB3 fusions are frequent in undifferentiated sarcomas of male children. *Mod Pathol.* 2015; 28:575–586. [PubMed: 25360585]
73. Li WS, et al. BCOR-CCNB3-positive soft tissue sarcoma with round-cell and spindle-cell histology: a series of four cases highlighting the pitfall of mimicking poorly differentiated synovial sarcoma. *Histopathology.* 2016; 69:792–801. [PubMed: 27228320]
74. Weinberg DN, Allis CD, Lu C. Oncogenic Mechanisms of Histone H3 Mutations. *Cold Spring Harb Perspect Med.* 2017; 7:a026443. [PubMed: 27864305]
75. Herz HM, et al. Histone H3 lysine-to-methionine mutants as a paradigm to study chromatin signaling. *Science.* 2014; 345:1065–1070. [PubMed: 25170156]
76. Lan F, Shi Y. Histone H3.3 and cancer: A potential reader connection. *Proc Natl Acad Sci USA.* 2015; 112:6814–6819. [PubMed: 25453099]
77. Behjati S, et al. Distinct H3F3A and H3F3B driver mutations define chondroblastoma and giant cell tumor of bone. *Nat Genet.* 2013; 45:1479–1482. [PubMed: 24162739]
78. Kervarrec T, et al. H3F3 mutation status of giant cell tumors of the bone, chondroblastomas and their mimics: a combined high resolution melting and pyrosequencing approach. *Mod Pathol.* 2017; 30:393–406. [PubMed: 28059095]
79. Cleven AHG, et al. Mutation Analysis of H3F3A and H3F3B as a Diagnostic Tool for Giant Cell Tumor of Bone and Chondroblastoma. *Am J Surg Pathol.* 2015; 39:1576–1583. [PubMed: 26457357]
80. Lüke J, et al. H3F3A mutation in giant cell tumour of the bone is detected by immunohistochemistry using a monoclonal antibody against the G34W mutated site of the histone H3.3 variant. *Histopathology.* 2017; doi: 10.1111/his.13190
81. Amary MF, et al. The H3F3 K36M mutant antibody is a sensitive and specific marker for the diagnosis of chondroblastoma. *Histopathology.* 2016; 69:121–127. [PubMed: 26844533]
82. Nielsen, GP., Rosenberg, AE. *Diagnostic Pathology: Bone.* Elsevier Health Sciences; 2017.
83. Wagner EJ, Carpenter PB. Understanding the language of Lys36 methylation at histone H3. *Nat Rev Mol Cell Biol.* 2012; 13:115–126. [PubMed: 22266761]
84. Lu C, et al. Histone H3K36 mutations promote sarcomagenesis through altered histone methylation landscape. *Science.* 2016; 352:844–849. [PubMed: 27174990]
85. Roux S, et al. RANK (receptor activator of nuclear factor kappa B) and RANK ligand are expressed in giant cell tumors of bone. *Am J Clin Pathol.* 2002; 117:210–216. [PubMed: 11863217]
86. Bjerke L, et al. Histone H3.3. mutations drive pediatric glioblastoma through upregulation of MYCN. *Cancer Discov.* 2013; 3:512–519. [PubMed: 23539269]

Figure 1. Chromatin and histone structure and a basic model for polycomb/SWI/SNF opposition
 (A) Beads on a string model of DNA packaging. DNA (line) is coiled around individual histone octamers to form a nucleosome. Each nucleosome contains a histone octamer consisting of 8 subunits: 2 each of H2A, H2B, H3 and H4 (right). The tails of each histone subunit may be modified with post-translational modifications that are either associated with active transcription (green dot) or transcriptional repression (red dot). (B) A general model for polycomb and SWI/SNF opposition. The SWI/SNF complex is associated with active histone modifications and a loose, open arrangement with fewer nucleosomes. Polycomb, on the other hand, is associated with dense nucleosome packing and repressive histone modifications. In a sarcoma with loss of function in SWI/SNF (malignant rhabdoid tumor and epithelioid sarcoma), the balance is shifted to favor polycomb repression and silencing of tumor suppressors. In sarcomas with polycomb loss (MPNST), the balance shifts to favor open, active chromatin and the expression of oncogenic drivers. Other sarcomas may have more complex effects on chromatin.

Figure 2. Immunohistochemical markers of altered epigenetic regulation

(A) This proximal-type epithelioid sarcoma exhibits loss of Ini1 (SMARCB1) expression by immunohistochemistry, which correlates with gene deletions in this member of the SWI/SNF chromatin remodeling complex. (B) Malignant peripheral nerve sheath tumors frequently have mutations or deletions in members of the polycomb repressive complex 2 leading to loss of the repressive H3K27me3 histone mark. This can be detected by immunohistochemistry, and is a specific marker of MPNST. (C) Chondroblastoma and (D) Giant cell tumor of bone harbor distinct recurrent mutations in genes encoding histone H3.3. immunohistochemical detection of the mutant proteins (H3F3B K36M mutant in chondroblastoma; H3F3A G34W mutant in GCT) is both sensitive and specific.

Figure 3.

Model for reorganization of histone modifications as a consequence of histone mutation. At top, two genes are depicted. Gene 1 shows a sharp peak of repressive histone modifications (e.g. H3K27me3) at the promoter (red peak) preventing gene transcription. Gene 2, has activating marks (e.g. H3K36me3) throughout the gene body (green) and is highly transcribed. An H3K36M mutation blocks H3K36 methylation, leading to loss of activating marks and gain in repressive H3K27me3. The gain of H3K27me3, however, is accompanied by a redistribution, with much deposited outside of promoter regions. Therefore, while there is overall increase in H3K27me3, the promoter of gene 1, actually shows a relative loss of H3K27me3 at the promoter and an increase in transcription. Gene 2, shows a decrease in transcription due to a relative increase in H3K27me3.

Table 1

Alterations in histone modifiers and histones in mesenchymal tumors

Tumor	Alteration
Malignant Rhabdoid Tumor	SMARCB1 mutations/deletions
Epithelioid Sarcoma	SMARCB1 mutations/deletions
Malignant Peripheral Nerve Sheath Tumor	SUZ12, EED mutations/deletions
Synovial Sarcoma	SS18(SYT)-SSX1, 2, 3 or 4 rearrangements
Ossifying Fibromyxoid tumor	Rearrangements including: EP400-PHF1, MEAF6-PHF1, EPC1-PHF1, ZC3H7B-BCOR, KDM2A-WWTR1, CREBBP-BCORL1
Endometrial stromal sarcoma (low-grade)	Rearrangements including: JAZF1-SUZ12, JAZF1-BCORL1, PHF1, MBTD1 rearrangements
Clear cell sarcoma of the kidney	BCOR internal tandem duplications, YWHAE-NUT2MB rearrangement
BCOR-rearranged sarcoma	BCOR-CCNB3 rearrangements (childhood); ZC3H7B-BCOR, BCOR-MAML3 (adult)
Undifferentiated round cell sarcoma	BCOR internal tandem duplication
Primitive myxoid mesenchymal tumor of infancy	BCOR internal tandem duplication
Giant cell tumor of bone	H3F3A mutation
Chondroblastoma	H3F3B mutation