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The epigenomics of sarcoma

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Abstract

Epigenetic regulation is critical to physiologic control of development, cell fate, cell proliferation, genomic integrity, and fundamentally, transcriptional regulation. This epigenetic control occurs at multiple levels including through DNA methylation, histone modification, nucleosome remodeling, and modulation of three-dimensional chromatin structure. Alterations in genes that encode chromatin regulators are common among mesenchymal neoplasms, a collection of more than 160 tumor types including over 60 malignant variants (sarcomas) that have unique and varied genetic, biologic, and clinical characteristics. Here, we review sarcomas in which chromatin pathway alterations drive disease biology. Specifically, we emphasize examples of dysregulation of each level of epigenetic control though mechanisms that include metabolic effects on enzymes that regulate DNA methylation and histone posttranslational modification, mutations in histone genes, subunit loss or fusions in chromatin remodeling and modifying complexes, and disruption of higher-order chromatin structure. Epigenetic mechanisms of tumorigenesis have been implicated in mesenchymal tumors ranging from chondroblastoma and giant cell tumor of bone to chondrosarcoma, malignant peripheral nerve sheath tumor, synovial sarcoma, epithelioid sarcoma and Ewing sarcoma: aggressive diseases which present in a younger patient population than most cancers. Finally, we review current and potential future approaches for the development of sarcoma therapies based on this emerging understanding of chromatin dysregulation.

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INTRODUCTION

Much of the focus of cancer biology has rightly emphasized cancer genetics owing to the observation that most cancers are driven by somatic genetic changes, ranging from viral introduction of an oncogene, to mutations that inappropriately activate or silence a gene, to copy number changes that amplify or remove a gene, or to rearrangements that aberrantly connect disparate sequences into a fusion transcript. Notably, a small but important fraction of these genetic changes physiologically dysregulate transcription across the whole genome. Herein enters the study of the epigenome, which is relevant in a number of cancer types, but appears particularly so in sarcomas, leukemias[1,2], and lymphomas[3,4] for reasons that remain unclear, but may be related to the mesenchymal and hematopoietic origins of these cancers, in contrast to carcinomas. The broad application of next generation sequencing provides additional granularity to our understanding of the alterations in chromatin regulators that can occur across myriad sarcoma histotypes.

Sarcoma is not a single entity, but rather a collection of more than 60 malignancies from within a broader set of over 160 different bone & soft tissue neoplasms, with diverse biologic and clinical characteristics. While the precise cell of origin is unknown for most sarcomas, sarcomas arise in tissues of mesenchymal lineage such as muscle, adipose tissue, and bone. Despite mesenchymal tissues making up the bulk of body mass, sarcomas are uncommon, comprising approximately 1% of human malignancies[5]. Clinically, sarcomas display a range of behavior from low grade tumors with minimal metastatic potential, to highly aggressive cancers with a tendency for widespread metastasis. For localized disease, surgery is typically the preferred treatment modality whereas chemotherapy or targeted therapy is used to treat metastatic disease[6]. The use of immunotherapy in sarcoma is largely still investigational, although immune checkpoint blockade is supported by expert guidelines in one common histotype (undifferentiated pleomorphic sarcoma)[6].

The genetics of sarcoma are highly variable. Some histotypes have relatively simple genetics and are driven by chromosomal translocations leading to fusion oncogenes, classically Ewing sarcoma (*EWSR1-FLI1*) and synovial sarcoma (*SSX-SS18*). Other sarcomas have complex genomic alterations (osteosarcoma), are driven by copy number alterations (well-differentiated/dedifferentiated liposarcoma), or by mutations in canonical drivers (gastrointestinal stromal tumors)[7].

Increasingly, many types of sarcoma have come to be considered as predominantly epigenetic diseases, with widespread epigenetic dysregulation initiated by a small number of, or even a single, genetic change[7,8]. The many translocation-associated sarcomas achieve substantial changes in the transcriptome of transformed cells from very few alterations in genomic DNA coding or copy number[9–17]. This fact in and of itself suggests that each of the fusion oncoproteins that associate with specific sarcoma subtypes taps into fundamental epigenomic mechanisms of transcriptome control.

For the purpose of this review, we will work from a specific definition of epigenetics. The meaning of the term has evolved over the decades and has been used in settings ranging from population genetics, to polygenic traits, to macromolecular structure, to the

molecular genetics of DNA methylation. Here, we will define epigenetics as non-sequence alterations (or at least non-local sequence changes) in the genome that impact transcriptional programs of downstream genes. Epigenetic regulation exists in multiple levels, for many of which there exist instructive examples of altered function in sarcomas (Figure 1, Table 1). Working outward from the DNA sequence itself, there are some sarcomas driven by alteration of gene and promoter DNA methylation, such as a subset of chondrosarcomas that are driven by *IDH* mutations[18]. As DNA is packaged by wrapping around nucleosomes, some secondary modifications of the histone proteins that comprise those nucleosomes represent a second layer of epigenetic control of transcription. Two recently elucidated examples in mesenchymal tumors that appear to be driven by somatic mutations in genes encoding histones ('oncohistones') are giant cell tumor of bone and chondroblastoma[19], although these and other histone mutations are observed in other sarcomas a well. The enzymatic complexes that act on nucleosomes provide a third layer of epigenetic complexity. These include enzymes that perform the 'writing', 'reading', or 'erasing' of posttranslational modifications (PTMs such as acetylation, methylation, phosphorylation, etc.) to histone proteins[20]. Malignant peripheral nerve sheath tumors are examples of sarcomas with alterations in a chromatin writing complex, whereas synovial sarcoma, malignant rhabdoid tumors, and epithelioid sarcoma are examples of sarcomas that are characterized by genetic alterations disrupting chromatin remodeling complexes involved in nucleosome repositioning or ejection (a fourth layer of complexity). Finally, the three-dimensional structure of chromatin (a fifth layer), with the creation of enhancer regions and their regulatory association with often distant genes, is a burgeoning area of investigation: FET (FUS, EWSR1, TAF15) domain proteins, the most common fusion partners in translocation-associated sarcoma fusion oncoproteins, are major drivers of phase-separation events that have been associated with these subnuclear organizational structures[21]. In this review, we will discuss how each of these five levels of epigenetic control can be lost in different sarcomas as a result of genetic alterations with an emphasis on how a better understanding of this dysregulation may provide opportunities for therapeutic intervention.

DNA METHYLATION

Using technologies based on microarrays and more recently reduced representation bisulfite sequencing, DNA methylation status, particularly 5-Me cytosine deposition at CpG islands, is currently one of the most heavily studied epigenetic changes in cancer. Research into DNA methylation is facilitated by its stability – patterns are retained biologically across cell states, and even after formalin-fixation and paraffin-embedding – as well as by its binary nature which lends itself to relatively straightforward bioinformatics. Ewing sarcoma, for example, has been shown to have a very distinct pattern of DNA methylation (including hypomethylation at enhancers targeted by the EWSR1-FLI1 oncoprotein) that clearly distinguishes it from other cancer types[22,23]. Indeed, even within the class of small blue round cell sarcomas, methylomes are subtype-specific and may have value in the differential diagnosis of histologically indistinguishable entities[24,25]. Methylation patterns also serve to distinguish among subtypes of rhabdomyosarcoma[26], nerve sheath tumors[27] and even within otherwise genetically homogeneous entities such as malignant rhabdoid tumors[28]. In dedifferentiated liposarcoma, specific methylation profiles correlate with

clinical outcomes[11]. In many cases, promoter elements are hypomethylated, while enhancers and coding sequences are hypermethylated, although the net consequences on transcription *in vivo* are not entirely predictable. Moreover, drugs that alter DNA methylation status with clear benefit in hematopoietic diseases have never found a role in sarcoma treatment, despite a long history of attempts[29]. This may be because of crosstalk between at least some layers of epigenetic regulation[30], that put DNA methylation changes downstream of the proximal or driving genetic alteration in these other layers (Figure 1), such as Polycomb deletions in malignant nerve sheath tumors, *SMARCB1* deletions in malignant rhabdoid tumors, and recently proposed phase transition effects in *EWSR1* fusion-driven sarcomas[31,32]. How such genetic alterations mechanistically alter the DNA methylome is an area of active research, in which there have been recent advances in at least one major class of sarcomas, the central chondrosarcomas.

Central Chondrosarcoma

The discovery of oncogenic mutations in the isocitrate dehydrogenase (IDH) enzymes provided fundamental new insights into the mechanisms by which metabolites regulate epigenetic marks and how dysregulation of cellular metabolism can drive oncogenesis[33–35]. Somatic mutations in cytosolic *IDH1* and mitochondrial *IDH2* are prevalent in acute myeloid leukemia (AML), glioma, cholangiocarcinoma, T cell lymphoma and several other tumor types[36]. Within sarcomas, *IDH* mutations are almost exclusively found in cartilaginous tumors, including 50–80% of central chondrosarcomas and in both their benign precursor (enchondromas) and in their advanced form (dedifferentiated chondrosarcomas)[18,37]. Somatic mosaic mutations in *IDH1* or *IDH2* underlie the pathogenesis of Ollier disease and Maffucci syndrome – non-hereditary diseases characterized by multiple enchondromas with increased risk of malignant transformation to chondrosarcoma[38,39]2.

IDH enzymes normally function to catalyze the NADP(H)-dependent interconversion of isocitrate and alpha-ketoglutarate (aKG)[36]. Cancer-associated *IDH* mutations occur at specific arginine residues within the catalytic sites of IDH1 (R132) and IDH2 (R172 and R140). These mutations disrupt normal enzyme function, while simultaneously conferring a neomorphic enzymatic activity that enables efficient reduction of aKG to the ‘oncometabolite’ 2-hydroxyglutarate (2HG)[40,41]. Given its structural similarity to aKG, 2HG acts as a competitive inhibitor of a large family of aKG-dependent dioxygenases with diverse biologic functions, including regulation of DNA hydroxymethylation, RNA demethylation, histone demethylation, DNA repair, and prolyl hydroxylation of collagen and hypoxia-inducible factors[36].

While the relative importance of different 2HG targets to oncogenesis remains an active area of investigation, 2HG-mediated inhibition of DNA and histone demethylation has been shown to play an important role in AML and glioma[42]. In particular, 2HG inhibits the TET DNA cytosine hydroxymethyltransferases and Jumonji histone demethylases resulting in increased methylation of DNA and histones, respectively[43–45]. Disruption of this metabolic–epigenetic axis leads to a repressive chromatin state that impairs expression of genetic programs required for normal differentiation and locks *IDH*-mutant cancer cells into

an undifferentiated state. In AML and glioma, small-molecule inhibitors of IDH-mutant enzymes can block 2HG production, reverse repressive chromatin states, and restore cellular differentiation[46–48]. The mutant IDH1 inhibitor ivosidenib and the mutant IDH2 inhibitor enasidenib were recently approved for the treatment of AML[49,50].

There have been more limited investigations into the mechanisms by which *IDH* mutations drive oncogenesis in chondrosarcoma. In mesenchymal stem/progenitor cells, mutant IDH-derived 2HG promotes hypermethylation of DNA CpG islands and increases repressive histone marks, resulting in impaired adipocyte and osteocyte differentiation *in vitro*[51–53]. The precise effects of mutant IDH and 2HG on the chondrocyte lineage are less clear, with impaired chondrocyte differentiation observed in mouse models but enhanced chondrocyte differentiation observed in human mesenchymal stem cells *in vitro*[51–54]. Introduction of mutant *IDH2* R172K into a mouse mesenchymal progenitor cell line resulted in loss of contact inhibition *in vitro* and was sufficient to promote growth of dedifferentiated tumor xenografts *in vivo*[53]. In contrast, a mouse model with mutant *IDH1* R132Q expression restricted to *Col2a1*-expressing chondrocytes exhibited multiple enchondroma-like lesions but no malignant progression to chondrosarcoma[54]. Notably, 2HG directly inhibits the aKG-dependent collagen prolyl 4-hydroxylase, resulting in impaired collagen maturation and defective extracellular matrix formation[55,56]; however, the importance of this 2HG-target in chondrosarcoma pathogenesis remains unknown.

In patients with chondrosarcoma, there has been no clear association between *IDH*-mutation status and clinical outcome, though a recent analysis of 89 chondrosarcoma cases, of which 41 were *IDH1* or *IDH2* mutated, showed an improved relapse-free and metastasis-free (though not overall) survival in subset of high grade chondrosarcoma bearing these mutations (22/47)[57,58]. The presence of *IDH* mutations in both primary and locally recurrent/metastatic lesions suggests that the *IDH* mutation may be an early event in chondrosarcoma pathogenesis[59]. The clinical phenotypes of Ollier disease and Maffucci syndrome indicate that *IDH* mutations may be sufficient for development of enchondromas but that additional genetic alterations may be required for progression to chondrosarcoma[38,39]. In clinical chondrosarcoma specimens, *IDH*-mutant tumors exhibit increased methylation of DNA CpG islands, but no clear association with histone methylation or DNA cytosine hydroxymethylation[53,57]. Unfortunately, treatment of *IDH*-mutant patient-derived chondrosarcoma cell lines with mutant-specific IDH inhibitors resulted in potent suppression of 2HG levels but did not reverse methylation of DNA/histones nor inhibit cell growth[51].

Notably, a different enzyme in the same tricarboxylic acid cycle pathway as IDH, succinate dehydrogenase (SDH), is implicated in the pathogenesis another sarcoma histotype. SDH deficiency, either as part of the Carney-Stratakis syndrome or in sporadic cases, is responsible for a subset of gastrointestinal stromal tumors (GIST) that lack more common KIT or PDGFRA mutations[60,61]. Loss of SDH activity results in an accumulation of succinate, which, similarly to 2HG, inhibits the activity of histone demethylases and the TET enzymes[62]. The latter leads to marked differences in DNA methylation profiles between SDH-deficient and KIT-mutated GIST[63]. These changes in DNA-methylation in SDH-deficient GIST have recently been linked to downstream alterations in 3D chromatin

structure through disruption of binding sites for the CTCF insulator[64]. Thus, there is an intriguing parallel between altered metabolism and epigenetic dysregulation in multiple rare sarcoma histotypes.

HISTONE MUTATIONS

Despite the relatively high frequency of driver mutations in chromatin modifying-enzymes in sarcomas and other cancers, it was only recently recognized that the fundamental substrates for this machinery, histone proteins, are also mutated in certain cancers (Figure 1). The first report of a histone driver mutation in cancer (i.e. an 'oncohistone') was in the setting of pediatric gliomas where they occur in two histone H3 variants, H3.3 or H3.1. Lysine to methionine mutations occur at lysine 27 in H3.1 or H3.3, and in H3.3 at glycine 34 with substitutions to arginine/valine[65,66] (Figure 2).

Shortly after the discovery of oncohistones in pediatric gliomas, methionine substitutions at lysine 36 in H3.3 (*H3F3B*) were reported in > 90% of chondroblastomas, and missense mutations (nearly all resulting in tryptophan substitution) were observed at glycine 34 in the *H3F3A* gene in > 90% of giant cell tumors of bone[67]. In the same study, lower frequency mutations also occurred in osteosarcoma (H3.3 G34R) and in chondrosarcoma (H3.3 K36M). Thus, oncohistones function as possible drivers in multiple mesenchymal lineages. Subsequent studies confirmed the presence of H3.3 G34 missense mutations in osteosarcoma and identified K36M/I mutations in H3.1, an H3 variant closely related to H3.3, in pediatric undifferentiated sarcoma[68–70].

The initial discovery of oncohistones has led to several important questions including why two different H3.3 genes (which encode identical proteins) are differentially mutated depending on the affected amino acid and cancer type, why specific oncohistone mutations occur at high frequency in tumors of mesenchymal lineage, whether these or other mutations occur in other cancers, and how oncohistones affect the local and long-distance chromatin landscape. While some of these questions remain unanswered, significant progress has been made in addressing the last two.

Through the work of several labs, the 'K-to-M' class of histone mutations has been shown to function as inhibitors of the cognate methyltransferases that normally act on lysine as a substrate leading to reorganization of the chromatin landscape[69,71,72]. In the case of H3.3 K36M, inhibition of the H3K36 methyltransferases NSD2 and SETD2 leads to the loss of H3K36 di- and tri-methylation and induces a gain in H3K27 methylation, particularly in intergenic regions[69]. As a result, the repressive PRC1 complex, which engages H3K27 trimethylation (H3K27me3), is titrated away from genes that are normally repressed, and redistributed to intergenic regions that contain aberrantly localized H3K27me3. The ultimate consequence of this reorganization is the expression of genes that are normally silenced during cell fate commitment thereby promoting the persistence of an undifferentiated progenitor population despite appropriate *in vitro* differentiation signals[69]. Accordingly, expression of H3.3 or H3.1 K36M in mesenchymal progenitor cells is sufficient to generate sarcoma-like tumors in a murine allograft model[69].

Interestingly, the ‘K-to-M’ paradigm commonly observed in oncohistone mutations has now been extended to the non-histone protein EZHIP. EZHIP contains a peptide sequence similar to the mutated region of the H3K27M oncohistone and is overexpressed in posterior fossa type A ependymomas where it inhibits PRC2[73–77]. Interestingly, EZHIP is also a fusion partner in a subset of endometrial stromal sarcomas, as discussed in the *Fusions with Polycomb Complex Components* section. Thus, the concept of ‘onco-histone mimicry’ broadens the mutational landscape underlying a variety of cancers.

In contrast, H3G34 mutations are less well understood. One notable difference is that mutations at H3G34 block H3K36 methylation exclusively in *cis* (i.e. on the same histone tail that harbors the mutations) while H3 K36M/I mutations also reduce H3K36 methylation in *trans* (i.e. on wildtype nucleosome tails)[69,71]. Inhibition of H3K36 methylation is hypothesized to result from impaired binding of the H3K36 substrate by SETD2, an H3K36 methyltransferase, due to H3G34 substitutions that introduce a bulky sidechain into a G33-G34 binding pocket near the active site of SETD2[78].

Despite differences in the *cis/trans* effects on oncohistone mutations at H3G34 and H3K36, both classes of oncohistone mutations have characteristics of oncogenic drivers. In which contexts effects in *cis*, which likely only affect local chromatin structure, versus effects in *trans*, which induce a global change in the chromatin landscape, are necessary to promote oncogenesis is an intriguing question that remains to be answered[69,72,79].

Since the identification of these first (i.e. ‘classical’) oncohistones, several groups have recognized other non-classical histone mutations in rare cases involving multiple cancer types[70,80–83]. These include AML, bladder cancer, uterine carcinosarcoma and mesenchymal tumors such as Ewing sarcoma, gastrointestinal stromal tumor, undifferentiated pleomorphic sarcoma, angiosarcoma, and desmoplastic small round cell tumor2. These non-classical histone mutations affect all four core histone families with frequent mutations in both the N-terminal tails, where the classical oncohistones mutants are located, and in the histone fold domains, which form the core of the nucleosome structure. The location of these novel oncohistone mutations is hypothesized to affect regulatory histone posttranslational modifications and/or nucleosome structure[70]. This may lead to downstream effects on chromatin-regulated processes including transcription and DNA repair. Additional work is ongoing to understand the potential function of these novel oncohistones in sarcomas and other tumor types.

HISTONE PTM ‘WRITER’ COMPLEXES

Malignant Peripheral Nerve Sheath Tumors

As discussed in the preceding section, the fundamental mechanism of two of the classical oncohistone mutations, H3.3 K27M and H3.3 K36M, is inhibition of histone PTM writer complexes. This paradigm extends from the histone proteins themselves to genetic alterations leading to loss of function in the enzyme complexes depositing the histone marks (Figure 3). Within sarcomas, malignant peripheral nerve sheath tumors (MPNST) are a classical example. The development of MPNST is associated with the germline syndrome of neurofibromatosis, which accounts for roughly half of cases[84]. The remainder

of cases occur either sporadically or more rarely as a result of prior radiotherapy, but in all settings commonly harbor precise somatic mutations in NF1 (82% (18/22) in one series)[85]. Efforts to better define the molecular events that lead to the development of MPNST identified mutually exclusive loss of function alterations in two core components of Polycomb Repressor Complex 2 (PRC2): SUZ12 (which can be codeleted with *NF1* by virtue of its adjacent location on 17q11.2) and EED[85,86].

PRC2 is responsible for depositing the repressive histone mark, H3K27me3, and has an important role in development and cell fate[87]. These genetic alterations in PRC2 components occur in the majority of NF1 associated, sporadic, and radiation-associated MPNST[85]. Notably, genetic alterations in a catalytic subunit of PRC2, EZH2, are observed in other cancers but not in MPNST[88]. As one would predict, SUZ12 or EED loss leads to suppressed PRC2 activity and decreased abundance of the PRC2-catalyzed histone mark, H3K27me3. Lack of H3K27me3 staining by immunohistochemistry is a biomarker that has been shown to be useful clinically in the pathologic evaluation of tumors suspected to be MPNST[89]. More recently, loss of H3K27 dimethylation has been suggested to be even more specific than H3K27me3 loss[90]. Because PRC2 is an important mediator of chromatin-regulated transcriptional repression, upregulation of gene expression is the most common transcriptional change observed in EED- and SUZ12-deficient MPNST compared to MPNST that are wildtype with respect to these genes[85]. Interestingly, the H3.3 K27M oncohistone mutation found in gliomas directly inhibits the EZH2 subunit of the PRC2 complex, suggesting that PRC2 loss of function may be a common mechanism for malignant transformation, but with important contextual or cell type-specific differences that are yet to be fully understood[71].

Fusions with Polycomb Complex Components

In addition to the loss of function alterations in PRC2 components seen in MPNST, polycomb complexes can also be perturbed in mesenchymal tumors through fusion events involving polycomb genes (Table 1). For instance, a member of the PRC1.1 complex, BCOR, is fused in a subset of small blue round cell tumors with a variety of different partners (e.g. BCOR-CCNB3) with considerable overlap in transcriptional programs induced by the different fusions[7,91–93]. The BCOR-PRC1.1 complex plays an important role in maintaining pluripotency in stem cell populations and suppresses mesodermal transcriptional programs; how sarcoma-associated BCOR fusions alter this function and contribute to sarcomagenesis is an ongoing area of research[94,95].

BCOR fusions are also identified in a subset of high-grade endometrial stromal sarcoma (ESS), and the PRC2 component, SUZ12, is fused to the transcriptional repressor JAZF1 in low-grade ESS[96–98]. The JAZF1-SUZ12 fusion reduces PRC2 activity and disrupts transcriptional repression[99]. In other ESS cases, JAZF1 is fused to PHF1, which targets the PRC2 complex to chromatin[100,101]. Finally, in yet another subset of ESS, the ‘oncohistone mimic’ EZHIP, which inhibits the catalytic activity of PRC2, is fused to a member of the NuA4 histone acetyltransferase complex, MBTD1[102,103]. This MBTD1-EZHIP fusion protein interacts with EZH2, the catalytic subunit of PRC2, and reduces the methyltransferase activity of the complex[76]. Taking the above together, we suggest that

impaired polycomb function may represent a common mechanism for tumorigenesis in a large fraction of ESSs.

CHROMATIN REMODELING COMPLEXES

Synovial Sarcoma

Synovial sarcoma, characterized by the SS18-SSX fusion, represents a model disease for the involvement of chromatin remodeling complexes in fusion oncoprotein-mediated sarcomagenesis. In mammals, the SWI/SNF family of multiprotein complexes are critical effectors of chromatin remodelling[104], existing in normal cells in at least three forms (Figure 4A): canonical BAF (BRG1/BRM Associated Factor, named after the ATPase components of the complex encoded respectively by the SMARCA4 and SMARCA2 genes), PBAF (Polybromo-associated BAF complex), and GBAF (GLTSCR1/1L-containing BAF, also known as non-canonical BAF or ncBAF). The amino terminal fusion partner, SS18, almost all of which is included in the chimeric oncoprotein, was identified as a frequent interactor with canonical BAF complexes shortly after its discovery[105–107]. Middeljans *et al.* were the first to report (in 2012) that SS18 was a stable member of canonical BAF (but not PBAF) complexes, and indeed that the t(X;18)-derived chimeric fusion oncoprotein, SS18-SSX, associates with the members of the BAF complexes in which native SS18 participates[108]. The mechanism by which SS18-SSX alters BAF complex function has since been interrogated through a variety of methods, revealing altered and disrupted associations. SS18-SSX can serve a bridging function connecting ATF2 to the PRC2 complex member TLE1 and in doing so represses the expression of important tumor suppressor genes including *CDKN2A* and *EGR1*[109–111] (Figure 4B). Another group identified a relationship with β-catenin resulting in the activations of specific target genes[112]. Incorporation of SS18-SSX into canonical BAF complexes was noted by Kadoch and Crabtree to result in the ejection of a member of the complex known to be a tumor suppressor, SMARCB1 (a.k.a. BAF47, hSNF5, INI1)[113] (Figure 4C). Although subsequent work has shown that synovial sarcoma-associated BAF complexes are quite distinct in their function from SMARCB1-absent BAF complexes[114], this altered membership of canonical BAF complexes is nonetheless likely important to disease biology not only in synovial sarcoma, but also in other mesenchymal tumors where SMARCB1 loss is a defining diagnostic feature[115], including the epithelioid variant of MPNST, poorly differentiated chordoma, malignant rhabdoid tumors and epithelioid sarcoma (discussed below).

Another subset of BAF complexes (that constitutively lack SMARCB1), termed GBAFs[116] (Figure 4A), comprise a functional dependency for both synovial sarcoma cells and cells of other cancer types such as acute myelogenous leukemia and malignant rhabdoid tumors. The functional dependency on BRD9, a stable member of GBAFs, was first reported in the target drive database[117], then followed up by multiple groups[118–120] (Figure 4C, bottom) (see Targeting Epigenetic Drivers in Sarcoma).

The distribution of BAFs across the epigenome has been reported to derive in part from an association of the SSX C-terminus with KDM2B and other members of the PRC1.1 complex[121]. As this complex normally functions to prime chromatin for subsequent transcriptional repression by PRC2, which BAF generally opposes, it is intriguing that the

fusion repurposes PRC1.1 as a localizing mechanism for BAF-mediated PRC2 antagonism at many target genes, leading to their activation (Figure 4D).

Epithelioid Sarcoma and Malignant Rhabdoid Tumor

Deficiencies in *SMARCB1* are characteristic of epithelioid sarcoma and malignant rhabdoid tumor, although there are notable differences between these entities. The age distribution for epithelioid sarcoma peaks in the adolescent-young adult population – younger than for most cancer patients but considerably older than for malignant rhabdoid tumor[122]. In terms of its primary site of disease, epithelioid sarcoma has an unusual predilection for superficial tissues in distal extremities; a less common “proximal” variant is based in the deep soft tissues of the limb girdle[123]. Rhabdoid cytomorphology is seen occasionally, particularly in proximal cases, but is not a diagnostic requirement[124]. Instead, epithelioid sarcoma typically shows a blend of spindled and epithelioid cells, all possessing enlarged nuclei with open chromatin. While the most reliable diagnostic marker in epithelioid sarcoma is loss of SMARCB1 protein expression in tumor nuclei[125], both epithelial (keratin, epithelial membrane antigen) and mesenchymal (vimentin, CD34) biomarkers are concurrently expressed, consistent with a polyphenotypic state of differentiation[126].

Whereas malignant rhabdoid tumor is characterized by bi-allelic deletion of *SMARCB1* on a particularly quiet genomic background[127], genome-wide studies of epithelioid sarcoma show a more prominent landscape of copy number alterations and a higher mutational burden (on par with e.g. glioblastoma multiforme), with SMARCB1 expression lost by several alternative mechanisms[128]. These include biallelic or monoallelic deletion of *SMARCB1* (which may be heterogeneous in the cell population) and/or overexpression of inhibitory miRNAs[128–131]. In transfected cell line models, SMARCB1 rescue leads to decreased proliferation and a reversal of polycomb-mediated repression, albeit not to as great an extent in epithelioid sarcoma as in malignant rhabdoid tumor cells[132]. In malignant rhabdoid tumors, recent work has shown the underlying mechanism of SMARCB1 loss to be associated with DNA methylation status and microenvironmental changes: homozygous large regional deletions of *SMARCB1* correlate with global hypomethylation and prominent cytotoxic T cell infiltration and immune checkpoint expression[133].

SWI/SNF complexes have roles not only in transcription, but also in DNA repair[134], with cBAF involved in the DNA end resection step of homologous recombination repair[135,136] and PBAF silencing transcription adjacent to double strand breaks[137]. Impaired SWI/SNF function can compromise DNA repair[138], which may explain the higher rate of genomic, abnormalities in epithelioid sarcoma in the context of a clinically more protracted tumor evolution than is seen in malignant rhabdoid tumors. Further supporting this concept are initial results from correlative sequencing studies on an epithelioid sarcoma clinical trial (NCT02601950)[139] which, while largely confirming prior genomic findings[128], also identified mutations in genes mediating DNA double strand break repair in a subset of epithelioid sarcomas (unpublished conference abstract)[140].

Rare sarcomas retain SMARCB1 but instead lose other members of the cBAF complex: SMARCA4, SMARCC1 or SMARCC2[141]. While *SMARCA4* deficiency may be an infrequent alternative to *SMARCB1* in epithelioid sarcoma, its loss via inactivating

mutation is the defining feature of a recently-discovered entity termed SMARCA4-deficient thoracic sarcomatoid tumors, considered by some to be sarcomas[142], while others have recently proposed that they represent a subset of smoking-associated, sarcomatoid lung carcinomas[143]. Usually presenting as mediastinal tumors in middle-aged males, these very aggressive cancers (median survival 6 months) can also show rhabdoid morphology, and typically lose expression not only of SMARCA4, but also of SMARCA2, while retaining SMARCB1[143]. A similar aggressive clinical course as well as molecular and histologic phenotype is also characteristic of small cell carcinoma of the ovary, hypercalcemic type[144], and the even more recently-recognized entity of SMARCA4-deficient undifferentiated uterine sarcomas[145,146]. Perhaps surprisingly, although these BAF-deficient sarcomas (including synovial sarcoma) share some apparent commonalities – such as a propensity for epithelial-mesenchymal biphasic differentiation, and evidence for a dependency on GBAF complexes localizing to CTCF sites proximal to promoters – they have very distinct transcriptional profiles[114]. While evidence suggests these cancers share induced dependencies on GBAF[117–120], the involved loci and consequent patterns of induced gene expression appear to be highly cell context-dependent.

HIGHER ORDER CHROMATIN ORGANIZATION

Ewing Sarcoma

There has recently been substantial progress in our understanding of the small family of proteins that become the amino terminus partners in more than half of known sarcoma-associated fusion oncoproteins. These FET domain proteins, namely FUS (previously TLS), EWSR1, and TAF15, all of which can interact with the SWI/SNF chromatin remodeling complex and demonstrate some capacity for interchangeable roles in sarcoma fusions[147], were initially poorly understood. By peptide sequence similarity, the three FET family members have an RNA binding domain, partly homologous to splicing factors, as well as a “transactivation” domain[148]. However, the target of the transactivating domain was unclear. Notably, from the native protein sequence only the transactivation domain is obligatorily included in the fusion oncoproteins[149]. An early observation was that this domain contains intrinsically disordered peptide sequences. When serine residues were experimentally substituted for the many tyrosines in these domains in EWSR1-FLI1, the transcriptional activation of target genes (including *NKX2–2*, *PRKCB* and *EZH2*) was abrogated[31]. Furthermore, both biochemical experiments with recombinant proteins or domains and experiments in cell lines showed FET proteins to be mediators of liquid-liquid phase separation (Figure 1E, panel 5; Box 1)[150–156]. These phase-separated droplets have also been demonstrated to interact with RNA Polymerase II and to organize transcription hubs, with much higher transcriptional outputs than traditional transcription factors binding to singular binding sites in promoters[32,157,158].

The ETS family of transcription factors that provide the C-terminal partners in Ewing sarcoma fusion oncoproteins contribute to the phase separation of chromatin by their binding (more significantly than to traditional singular ETS consensus binding sequences in promoters) to multimeric sites present in microsatellite regions with many GGAA repeats[159]. The multimeric associations between multiple contiguous binding sites on

chromatin with the low-energy but multiplied prion-like domain interactions of EWSR1-ETS fusions generate novel superenhancers in these microsatellite regions, often regulating the transcription of genes located at a great linear distance via higher order chromatin looping[31,32,160]. When the EWSR1-FLI fusion oncoprotein is depleted in Ewing sarcoma cells, changes in the epigenome at promoters, enhancers, and super-enhancers imply an important role for the fusion in regulating the epigenetic landscape[161].

These transcription hubs and target genes associated by three-dimensional chromatin structure have implications for nucleosome distribution and specific histone marks, as well as with the chromatin remodeling complexes that enzymatically drive these changes in epigenomic structure and function. SWI/SNF or BAF complexes are involved in the ejection of nucleosomes from enhancer elements and have been found to associate with both these native FET protein transcription hubs as well as with sarcomagenic fusion oncoproteins[31,147].

Interestingly, the binding pattern of BAF complexes that incorporate SS18-SSX in synovial sarcoma suggests that these may also participate in some types of phase-separated transcription hubs, because the BAF enrichment patterns on ChIPseq broaden from the typical promoters and enhancers[114], to include more of the gene bodies of target loci.

CLINICAL TRANSLATION: TARGETING EPIGENETIC DRIVERS IN SARCOMA

Although not the focus of this review, it is worth noting that insights into sarcoma biology from epigenetics-driven research have rapidly impacted clinical diagnostics. Histone mutation-specific antibodies exist: H3FA G34W immunohistochemistry has value in the diagnosis of difficult or malignant cases of giant cell tumor of bone[162]. As mentioned above, nuclei demonstrating loss of di- or tri- methylated H3K27 help define MPNST[89,90], while loss of SMARCB1 or SMARCA4 can seal the diagnosis of the epithelioid variant of MPNST, epithelioid sarcoma, chordoma, malignant rhabdoid tumor and related thoracic and gynecologic malignancies (all entities that were especially problematic for pathologists before these tools became available)[115]. Mutation-specific PCR has value to identify *IDH1/2* mutations in dedifferentiated chondrosarcoma[163], and karyotyping, FISH or RT-PCR have historically aided the diagnosis of fusion oncogene sarcomas (including not only Ewing sarcoma, but a growing list of additional entities bearing *EWSR1*, *FUS* or other translocations)[164]. Such methods are now being replaced by more modern multiplexed hybrid capture sequencing, anchored PCR or color coded probe pair technologies to identify most fusion events with a single assay[165–167]. Finally, DNA methylation arrays are emerging as a pan sarcoma diagnostic tool based on the recognition of sarcoma subtype-specific methylomes[23,24]. “Liquid biopsy” approaches are in advanced states of translational research, but have yet to enter routine clinical practice for epigenetically-driven sarcomas[168].

The identification of the genetic events that lead to downstream epigenetic alterations in some sarcomas has opened the door for targeted therapeutic interventions. This has been made possible by 1) an understanding of the detailed nature of the chromatin alterations that occur in the setting of the genetic defect, and 2) the development of second-generation

chromatin targeting drugs. The latter has moved the field beyond early HDAC and DNA methyltransferase inhibitors; emerging classes of small molecule epigenetic drugs hold promise for manipulating the activity of chromatin-modifying enzymes with a level of specificity not previously feasible[169,170].

Despite this progress, the majority of chromatin pathway alterations in sarcoma are not sufficiently understood to develop targeted therapeutic approaches, although there are several notable exceptions (Table 1). For example, epithelioid sarcoma and malignant rhabdoid tumor, as discussed above, are characterized by loss of the SWI/SNF (BAF) remodeling complex subunit SMARCB1, which interacts with a regulatory interface of the nucleosome (the acidic patch) through its C-terminal domain[171–173]. Loss of SMARCB1 induces dependency on the PRC2 methyltransferase complex which has subsequently been targeted via inhibition of its catalytic subunit EZH2[174,175]. Similarly, SMARCA4-deficient neoplasms show evidence for unopposed Polycomb activity with resulting sensitivity to EZH2 inhibitors[176]. A phase 2 study (NCT02601950)[139] of the EZH2 inhibitor tazemetostat included advanced/metastatic epithelioid sarcoma patients; initial results (disease control in 10 / 31 patients) exceeded prespecified criteria for success and led to a doubling of the cohort size. As existing sarcoma drugs (cytotoxic chemotherapy and tyrosine kinase inhibitors) confer minimal benefit in epithelioid sarcoma, these phase 2 findings led to approval by the FDA in 2020. Updated results (unpublished conference abstract)[177] on the final cohort of 62 patients showed disease control in 16 (26%) and objective responses in 9 (15%), which is better than what had been achieved in epithelioid sarcoma with existing chemotherapy regimens. Interestingly, EZH2 inhibition has not been effective clinically in synovial sarcoma (unpublished conference abstract)[178], perhaps owing to the fact that the SSX-SS18 fusion protein forms a BAF complex with altered composition and aberrant activity instead of loss of function[113,121].

Two additional examples of potential mechanism-based targeting of chromatin pathway alterations in sarcoma include IDH-mutated chondrosarcoma, and targetable dependencies of the (as yet untargetable) SS18-SSX fusion oncoprotein in synovial sarcoma. Inhibitors of IDH1 (ivosidenib) and IDH2 (enasidenib) have been developed in the setting of IDH-mutant acute myeloid leukemia and are now being evaluated in clinical trials (e.g. NCT02273739, NCT02073994)[179,180] for the treatment of the subset of chondrosarcomas that harbor IDH1 or 2 mutations[181]. A recent a Phase I study of IDH1 inhibition in IDH1-mutant chondrosarcomas suggest a potential but modest benefit in the conventional, but not dedifferentiated, subtype[182]. Two hypotheses for this subtype-specific difference are that the dedifferentiation leads to IDH1-independent growth or that the changes to chromatin environment are ‘locked-in’ and cannot be reversed by a decrease in the oncometabolite. The final results of this and other clinical trials[183] will be informative in evaluating the efficacy of this approach.

In synovial sarcoma and malignant rhabdoid tumors, recent preclinical data demonstrate a possible role for targeting the bromodomain containing protein, BRD9, a GBAF subunit as described above (Figure 4)[119,184]. Loss of SMARCB1 leads to compensatory use of alternative SWI/SNF (BAF) complexes, such as those incorporating SMARC paralogs and BRD9, with associated widespread changes in gene expression (including those controlling

stem cell differentiation) and CTCF-regulated higher-order chromatin structure[120,132]. BRD9 can be targeted with emerging agents[119], although those most effective *in vitro* (chemical degraders) have delivery and toxicity issues that are necessitating further pharmacological development before they can be used clinically. Additional work is needed to validate BRD9 as a target, and to develop compounds that can safely target this protein clinically.

Future successful therapeutic strategies in sarcomas with chromatin driver mutations will likely result from targeting either induced functional dependencies or through a reversal of one or more elements of the pathologically altered chromatin landscape. A hypothetical example of the latter is to leverage the reciprocal nature of multiple posttranslational modifications (PTMs), which have different functions but occur at the same residue. The aberrant gain in one PTM because of a loss of the reciprocal mark due to a genetic lesion in the ‘writer’ complex could be targeted by inhibiting the ‘writer’ or ‘reader’ of the inappropriately gained mark. One setting for exploring this approach is in those subsets of MPNST and endometrial stromal sarcoma which have mutations in the H3K27me3 writer complex, PRC2, since H3K27me3 and H3K27ac are reciprocal and have transcriptionally repressive and activating functions, respectively[113,119,120,185]. For instance, inhibition of the H3K27 acetyltransferase, p300, or H3K27ac reader domain containing proteins such as BRD4 could be potential strategies in PRC2 deficient sarcomas. Combining inhibition of the BET family of histone PTM readers and targeted degradation of one BET family member, BRD4, has also been proposed as a synthetic lethal strategy in MPNST[186].

One challenge in designing targeted therapies for sarcomas with chromatin pathway drivers is that chromatin regulators have diverse and incompletely understood functions, which complicates efforts to understand their pathological dysregulation let alone the activity of small molecules targeting them. Pursuing therapies in this setting may be more complex than studying inhibitors of signaling pathway drivers where at least the proximal downstream events are better characterized. This challenge is particularly pronounced in the setting of sarcomas with complex genomics such as undifferentiated pleomorphic sarcoma, where a subset harbor heterogeneous chromatin pathway alterations without a defined driver[11]. The first step to developing epigenetic therapeutics in these diseases is to identify which chromatin pathway genetic alterations affect the epigenome. Further investigations into which of these alterations affect chromatin regulated processes such as transcription, control of antitumor immunity, differentiation, and DNA damage repair will be needed. Having thus defined ‘functional’ alterations, selection and pre-clinical testing of targeted therapies can follow paradigms similar to sarcomas with clearly defined epigenetic drivers.

Another potential challenge is that chromatin changes resulting from mutations in chromatin regulators may be relatively difficult to reverse, which could explain the modest response rates of EZH2 inhibition in epithelioid sarcoma and IDH1 inhibition in chondrosarcoma. Combinations of chromatin targeting drugs may be needed to ultimately reverse the effects of chromatin dysregulation in these sarcomas.

That said, we are optimistic that in depth and rigorous investigations of both normal and aberrant chromatin-modifying enzyme complexes will continue to inform efforts to

therapeutically intervene in epigenetically-driven sarcomas. We also suggest that because of this complexity, therapeutically targeting tumors driven by derangements of chromatin regulation may represent an ideal opportunity to revisit the strategy of phenotypic drug discovery, which has fallen out of favor in the era of great success in targeted therapies but may be one valuable approach to in addition to the target-based strategies described above.

CONCLUSIONS

As this review demonstrates, there has been a remarkable evolution in our appreciation of the key role of epigenetic dysregulation in many if not most sarcomas. It is humbling to recall that, historically, targeted sequencing approaches focusing on proliferation and apoptosis-related "cancer genes" in sarcomas (and gliomas) failed to include many genes involved in epigenetic control[9,187] and thus, for instance, *IDH1* mutations were therefore instead first identified by a whole exome approach in gliomas[33]. Even to this day, the conventional precision oncology approach of matching kinase inhibitors to genetic alterations activating signaling through the MAPK pathway has been largely disappointing in sarcomas (with a few remarkable exceptions such as gastrointestinal stromal tumor[188], and NTRK fusion-driven sarcomas[189–191]). Thus, further clinical progress in targeting epigenetic dysregulation in sarcomas will depend on expanded clinical genomic testing that includes genes involved in epigenetic pathways as well as robust profiling of DNA methylation and histone modifications carefully paired with new agents that can specifically target these aberrant epigenetic states.

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GLOSSARY

Chondrosarcomas
A malignant cartilaginous matrix-producing tumor often driven by IDH1/2 mutations. Typically arising in the axial skeleton of middle-aged patients, these sarcomas can be slow growing but resistant to existing systemic therapy and radiotherapy.

Nucleosomes
Basic repeating structural units of the chromosome consisting of eight histone proteins (2 each of four core histones, H3, H4, H2A, and H2B) and 147 base pairs of DNA wrapping the structure.

Giant cell tumor of bone
A benign but often locally aggressive neoplasm of bone in young adults driven by missense histone mutations at H3.3G34. These tumors have a propensity for local recurrence and present as destructive, radiolytic lesions that destroy bone underneath articular surfaces.

Chondroblastoma

A benign cartilaginous neoplasm that characteristically arises at the ends of the body's long bones, close to the joints, and occurs predominantly in adolescents. Chondroblastoma is driven by the H3.3K36M histone mutation.

Malignant peripheral nerve sheath tumors
Sarcomas arising within peripheral nerves, about half of which are sporadic and half which occur in the context of congenital *NF1* inactivating mutations (neurofibromatosis type 1). Development of malignant peripheral nerve sheath tumors is additionally driven by (mutually exclusive) loss of function alterations in *SUZ12* or *EED*.

Synovial sarcoma
A malignant translocation-associated sarcoma driven by the *SS18-SSX* gene fusion. Although frequently arising in extremities near joints, the term is a misnomer as the cell of origin is unknown and the tumor is not derived from synovium, nor does it differentiate into synovial-type tissue. Exists in monophasic spindle cell forms or as a biphasic type with areas of epithelial differentiation.

Malignant rhabdoid tumors
Highly aggressive, malignant tumors that occur in infants and young children. Three presentations exist: kidney, extrarenal, and brain (termed atypical teratoid/rhabdoid tumor); all are characterized by bi-allelic deletion of *SMARCB1*.

Epithelioid sarcoma
Malignant soft tissue sarcoma in distal extremities, with mixed features of mesenchymal and epithelial differentiation. Typically affects adolescents and young adults, metastasizes aggressively and is resistant to conventional chemotherapies. Characterized by inactivating mutations in *SMARCB1*.

Chromatin remodeling complexes
Multiple families of protein complexes that alter chromatin structure to regulate gene expression. Their functions include alteration of nucleosome assembly (maturation and spacing), chromatin access (nucleosome repositioning or ejection), and nucleosome editing (histone exchange or eviction).

Phase separation
A physical process in which a single homogenous liquid phase spontaneously separates into two distinct phases due to changes in environment such as pH, temperature, salt and protein concentration.

Bisulfite sequencing
Current gold standard sequencing strategy for detecting DNA methylation based on the conversion of unmethylated cytosine to uracil after treatment with sodium bisulfite (without modification of methylated cytosine).

CpG islands
Segments of genomic DNA, several hundred base pairs in length, that contain a large number of CpG dinucleotide repeats. When occurring near promoters of expressed genes,

CpG islands are usually unmethylated; in contrast, CpG dinucleotides occurring in other contexts tend to be methylated.

Ewing sarcoma
A malignant bone or soft tissue tumor comprised of uniform small blue round cells, typically affecting children and adolescents. Driven by chromosomal translocations resulting in transcripts fusing FET (*FUS*/*EWSR1*/*TAF15*) genes with ETS family transcription factors, *EWSR1-FLI1* is the most common variant.

Enhancers
Gene regulatory elements that bind transcription factors and cofactors to activate transcription of target genes that may be located a relatively great linear distance away, and independently of their orientation on DNA.

Polycomb
A group of proteins originally discovered in *Drosophila* involved in establishment and maintenance of developmental gene expression programs through formation of PRC complexes that repress gene expression by methylation of histone H3K27 (PRC2) and ubiquitination of H2A119 (PRC1).

Gastrointestinal stromal tumor
Mesenchymal neoplasm of the gastrointestinal tract, derived from the interstitial cells of Cajal. Activating mutations in the *KIT* (or *PDGFRA*) receptor tyrosine kinases are the key initiating oncogenic events in the majority of cases, making imatinib and related tyrosine kinase inhibitors an effective targeted therapy for this disease.

Undifferentiated pleomorphic sarcoma
A malignant mesenchymal tumor of undefined histogenesis, histologically characterized high grade spindle cells producing a nonspecific collagenous matrix. Previously termed malignant fibrous histiocytoma and considered a diagnosis of exclusion.

Angiosarcoma
An aggressive, malignant endothelial cell tumor of vascular or lymphatic origin that can arise anywhere in the body, sporadically or sometimes in association with radiation exposure or lymphedema. Angiosarcomas are especially infiltrative and prone to metastatic spread.

Desmoplastic small round cell tumor
An aggressive, malignant neoplasm that typically presents as a large mass in the abdomen of adolescent and young males. Characterized by a translocation resulting in *EWSR1-WT1* fusion transcripts, this sarcoma does not respond well to any currently available systemic therapies.

Endometrial stromal sarcoma
A type of uterine malignancy with low and high grade forms that are associated with distinct genetic rearrangements and fusion oncogenes. Typically presenting in middle age, the disease is relatively slower to progress than most other types of sarcoma.

Sarcomatoid tumors

A descriptive term for neoplasms of non-mesenchymal origin that develop a sarcoma-like histologic phenotype (characterized by spindle cell cytomorphology, matrix production and cell-matrix interactions) – for example, carcinomas that have undergone epithelial-mesenchymal transition.

Mediastinal tumors
A term for primary neoplasms of the thoracic cavity, other than lung cancers.

Superenhancers
Clusters of enhancers in close genomic proximity with high concentrations of bound transcriptional co-activators that control expression programs to regulate cell identity.

Chromatin looping
A model for long-range control of gene expression to allow for direct contact of promoters and enhancers over long linear distances by looping out the intervening chromatin. Loops are mediated and stabilized by proteins and complexes including CCCTC-binding factor (CTCF), Mediator, and Cohesin.

Chemical degrader
A class of compounds that bind a target protein through one chemical domain and through a second domain recruit the cereblon E3 ubiquitin ligase complex leading to degradation of the target protein. Degradation can have a distinct biologic effect from small molecule inhibition of the target in cases where the target protein has non-enzymatic functions.

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BOX 1:

Liquid-liquid phase separation

Phase separation is a physical process for liquid-liquid demixing by which a supersaturated solution of components spontaneously separates into two distinct but stable phases, a high-concentration phase and a low-concentration phase[192–194]. An everyday example of would be the demixing, or phase separation, of immiscible fluids such as oil and water.

While phase separation is well-known in polymer science[195], the concept of phase separation as a possible mechanism for membraneless compartmentalization and spatiotemporal regulation of biological reactions is a recent development in biology[192–194,196]. These membraneless compartments, recently termed biomolecular condensates[193,194], are highly diverse in molecular composition, subcellular localization and functions, and include subnuclear bodies such as nucleoli, Cajal bodies, PML nuclear bodies and nuclear speckles[197], as well as cytoplasmic structures such as stress granules and P-bodies[198].

Formation of biomolecular condensates is driven by multivalent protein-protein or protein-RNA interactions[199,200] involving two major classes of proteins that can phase separate under physiological conditions. The first class of proteins contain multiple folded domains or modules that frequently interact with linear motifs of other proteins, with increasing number of modules conferring higher propensity to phase separate, and is exemplified by the clustering of signaling molecules to facilitate signal transduction[199,201,202].

The second class of proteins contain intrinsically disordered regions which lack a defined 3-dimensional structure and are typically enriched in low complexity domains – repeat sequences of a limited number of amino acid residues that drive phase separation, such as asparagine, glycine, glutamine, serine, arginine, lysine, aspartic acid, glutamic acid, phenylalanine and tyrosine[203–205]. For example, the ~30 members of the FUS family of proteins (including the sarcoma-associated FET proteins FUS, EWSR1 and TAF15) share similar domain structures[206], and undergo phase separation primarily by interactions between arginine and tyrosine residues[200].

There is increasing evidence that transcription factors mechanistically activate genes through phase separation[207], and that aberrant formation of biomolecular condensates is associated with neurodegenerative diseases and cancer[194,208]. Nevertheless, in the rapidly emerging field of liquid-liquid phase separation biology, it should be noted that while many proteins have been shown in elegant studies to phase separate *in vitro*, extrapolating or experimentally determining their *in vivo* functional consequences has proven far more challenging[209,210].

Figure 1 |. Schematic of five layers of epigenomics that drive transcriptional programs in sarcomas.

For Figure 1, please refer to Figure 1 of the manuscript Nacev BA, Jones KB, Intlekofer AM, Yu JSE, Allis CD, Tap WD, Ladanyi M, Nielsen TO. The epigenomics of sarcoma. Nat Rev Cancer. 2020 Oct;20(10):608–623. doi: 10.1038/s41568-020-0288-4. Epub 2020 Aug 11. PMID: 32782366.

Figure 2 |. Classical oncohistone mutations alter histone PTM ‘writer’ complex activity:
For Figure 2, please refer to Figure 2 of the manuscript Nacev BA, Jones KB, Intlekofer AM, Yu JSE, Allis CD, Tap WD, Ladanyi M, Nielsen TO. The epigenomics of sarcoma. Nat Rev Cancer. 2020 Oct;20(10):608–623. doi: 10.1038/s41568-020-0288-4. Epub 2020 Aug 11. PMID: 32782366.

Figure 3 |. Genetic alterations in histone PTM ‘writer’ complex components are found in various sarcoma subtypes.

For Figure 3, please refer to Figure 3 of the manuscript Nacev BA, Jones KB, Intlekofer AM, Yu JSE, Allis CD, Tap WD, Ladanyi M, Nielsen TO. The epigenomics of sarcoma. Nat Rev Cancer. 2020 Oct;20(10):608–623. doi: 10.1038/s41568-020-0288-4. Epub 2020 Aug 11. PMID: 32782366.

Figure 4 |. Mechanisms of action of SS18-SSX in synovial sarcoma.

For Figure 4, please refer to Figure 4 of the manuscript Nacev BA, Jones KB, Intlekofer AM, Yu JSE, Allis CD, Tap WD, Ladanyi M, Nielsen TO. The epigenomics of sarcoma. Nat Rev Cancer. 2020 Oct;20(10):608–623. doi: 10.1038/s41568-020-0288-4. Epub 2020 Aug 11. PMID: 32782366.

Table 1 |

Chromatin pathway mutations in sarcoma

Histotype	Genetic alteration	Epigenetic role of altered pathway	Investigational targeted therapy
DNA methylation			
Chondrosarcoma	*IDH1/IDH2* neomorphs	Oncometabolite → alteration of DNA methylation and Histone modification	ivosidenib (NCT02073994)[180], enasidenib (NCT02273739)[179]
Histones			
Giant cell tumor of bone (> 90%), chondroblastoma (> 90%), chondrosarcoma, osteosarcoma, UPS	Histone H3 K36 or G34 mutations	Common substrate for chromatin regulation	Pressing need
Histones PTM 'writers'			
MPNST	*SUZ12* or *EED* loss of function	Histone PTM 'Writer'	Pressing need
		PRC2	
BCOR-Rearranged	*BCOR-CCNB3*	Histone PTM 'Writer'	Pressing need
small blue round cell tumors	*BCOR-MAML3*	non-canonical PRC1	
	ZC3H7B-BCOR		
	KMT2D-BCOR		
Endometrial stroma sarcoma	*JAZF1-SUZ12*	Histone PTM 'Writer'	Pressing need
	JAZF1-PHF1	PRC2; non-canonical PRC1	
	MBTD1-EZHIP		
	ZC3H7B-BCOR		
Chromatin remodeling complexes			
Epithelioid sarcoma	SMARCB1 loss	Remodeling	tazemetostat (FDA approved, 2020)
Malignant rhabdoid tumor	SMARCB1 loss	Remodeling	EZH2 inhibitors (NCT02601937; NCT03213665; NCT02875548)[211–213]
Synovial sarcoma	SSX-SS18 fusion	Remodeling	Brd9 inhibitors
Higher order chromatin organization			
FUS/EWSR1/TAF15 translocation-associated sarcomas	FUS/EWSR1/TAF15-associated fusions	Three-dimensional chromatin structure	Pressing need