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## Disruption of mammalian SWI/SNF and polycomb complexes in human sarcomas: mechanisms and therapeutic opportunities

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### Abstract

Soft-tissue sarcomas are increasingly characterized and subclassified by genetic abnormalities that represent underlying drivers of their pathology. Hallmark tumor suppressor gene mutations and pathognomonic gene fusions collectively account for approximately one-third of all sarcomas. These genetic abnormalities most often result in global transcriptional misregulation via disruption of protein regulatory complexes which govern chromatin architecture. Specifically, alterations to mammalian SWI/SNF (mSWI/SNF or BAF) ATP-dependent chromatin remodeling complexes and polycomb repressive complexes cause disease-specific changes in chromatin architecture and gene expression across a number of sarcoma subtypes. Understanding the functions of chromatin regulatory complexes and the mechanisms underpinning their roles in oncogenesis will be required for the design and development of new therapeutic strategies in sarcomas.

### Keywords

soft-tissue sarcoma; epigenetic regulation; chromatin remodeling; mSWI/SNF complexes; BAF complexes; polycomb repressive complexes; fusion oncoproteins, oncogenesis

### Introduction

Sarcomas are rare, highly aggressive malignancies marked by the formation of solid tumors in soft tissues or bone that are derived from cells of mesenchymal origin. Sarcoma subtypes possess diverse genetic profiles ranging from a single pathognomonic genetic event, such as chromosomal translocations in synovial sarcoma and Ewing sarcoma, to extensive mutational burden and complex genomes [1–7]. Sarcomas arise in all age groups, but are disproportionately present in children, accounting for a significant number of cancer-related mortalities in both pediatric and young adult patients [8]. In the absence of molecularly

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targeted therapies, treatment of sarcomas most often relies on cytotoxic chemotherapy in combination with radiation and surgery [9–11]. Upon treatment with these traditional approaches, patients with cases of localized sarcomas achieve 5-year event-free survival rates of 60–70% [12,13]. However, these aggressive malignancies are prone to metastasize, particularly to the lungs, and survival rates for patients with metastatic disease are 20–25% at 2 years [14,15]. Improved patient outcomes rely on the development of targeted therapies tailored to counter the transformative oncogenic activity of the underlying genetic alterations driving each sarcoma subtype. The development of such therapies requires a detailed molecular understanding of the mechanism by which certain genetic perturbations are capable of co-opting specific tissue environments to activate cellular proliferation programs that facilitate solid tumor growth. This review explores the current understanding of recurrent genetic alterations to epigenetic transcriptional regulators across a set of molecularly well-characterized sarcoma subtypes and proposes clinical strategies for reversing their oncogenic capabilities (Table 1).

## Epigenetic gene regulation

Genetic events that perturb the protein machinery responsible for epigenetic regulation can drive widespread changes in transcriptional programs. The human genome consists of approximately 3 billion base pairs of DNA compacted in the nucleus of each cell through the organized wrapping of 146 DNA base pairs around histone protein octamers which form nucleosomes [16–18]. Nucleosomes pack tightly against one another to facilitate the storage of genetic information and in doing so form compacted chromatin. This compaction provides structural integrity and serves as a physical barrier to safeguard the DNA content from genetic alterations (i.e. mutations). This mechanism of DNA compaction sterically inhibits protein machineries involved in transcription, replication, recombination, and DNA repair from accessing and binding to DNA. Consequently, the tight compaction of DNA must be relaxed in a highly controlled manner to facilitate access to specific gene elements, such as gene promoters by transcription factors and RNA Pol II, to permit appropriate gene transcription and other essential cellular processes to occur.

The regulation of chromatin architecture, required for proper lineage-specific gene expression, occurs by controlling the DNA–histone interactions through a number of mechanisms that include DNA methylation, covalent modification of histones, and ATP-dependent chromatin remodeling. Control of DNA accessibility to maintain the precise balance between transcriptional activation and repression is achieved through the enzymatic activities of large multi-subunit protein complexes. Two such classes of complexes form the basis of this review, and include the mammalian switch/sucrose non-fermentable (SWI/SNF) complexes and the polycomb group (PcG) protein complexes. SWI/SNF complexes are trithorax group (TrxG) protein members that utilize the power of ATP hydrolysis to carry out the energetically unfavorable process of remodeling nucleosome–DNA interactions to facilitate DNA accessibility [19,20]. This activity is antagonistic to forms of PcG protein complexes that covalently modify the N-terminal tails of histone proteins post-translation to increase chromatin compaction associated with transcriptional repression [20–24]. The precise balance between SWI/SNF and PcG function is tailored to the specific transcriptional needs of different cells through differentiation and development. In

embryonic stem (ES) cells, PcG proteins bind cell lineage-specific genes to repress transcription and prevent untimely activation of differentiation pathways [25,26]. SWI/SNF complex activity is critically important for activating transcription of lineage-specific genes to promote cell differentiation [27,28]. In terminally differentiated cells, PcG proteins continue to repress genes of other tissue lineages to maintain the cellular identity [29]. This careful balance between the activity of SWI/SNF and PcG protein complexes facilitates the accessibility and expression of genes required for differentiation of a progenitor cell into a cell of committed lineage, while maintaining repression of genes involved in alternative cell fates.

## Composition and function of mSWI/SNF (BAF) complexes

The TrxG proteins were first discovered and characterized in *Drosophila*, in which they are required for the activation of *Hox* genes and others for proper body segmentation [30–35]. In mammals, TrxG proteins are a diverse and heterogeneous group involved in the transcriptional activation of genes through a number of chromatin modifying activities. TrxG protein members include the SWI/SNF family of chromatin remodeling complexes that possess an ATPase to harness the power of ATP to modify the nucleosome landscape in a variety of manners.

SWI/SNF complexes were initially discovered via the identification of redundant phenotypes upon mutation of genes involved in mating type switching (SWI) and nutrient switching (SNF) regulation in yeast [36,37]. Mutation of histone coding genes suppressed these phenotypes, indicating that these SWI/SNF proteins were involved in altering chromatin structure to regulate transcription [38]. Subsequently, purification in yeast produced an ~1MDa complex consisting of 9–12 subunits that was capable of disrupting mono-nucleosomes *in vitro* [39–41]. The SWI/SNF family of proteins is evolutionarily conserved with homologous members in flies, plants, and mammals [19]. The mammalian SWI/SNF (mSWI/SNF) complexes have a greater number of subunits, up to 15 subunits in a given complex assembly, to scale with the greater demand for transcriptional regulation and enable specialized cellular functions in multicellular organisms. Furthermore, there are at least two distinct populations of mSWI/SNF complexes, named BAF (BRG1 or BRM-associated factors) and PBAF (polybromo-associated BAF), characterized by differential subunit compositions (Figure 1A) [42]. Both complexes possess a catalytic ATPase subunit of either BRG1 or BRM, along with other core subunits (BAF155, BAF170, BAF47, BAF57), but differ in their ARID domain-containing subunit (BAF250A/B in BAF and BAF200 in PBAF) and the incorporation of the BAF180, BRD7, and BAF45A subunits specifically in PBAF complexes. To allow for further specialization of transcriptional regulatory capacity of mSWI/SNF complexes, a number of subunit members possess multiple paralogs with tissue-specific expression that play a role in directing ES cells toward terminal differentiation, such as in neuronal and cardiac differentiation [43–46]. The diversity of subunit members provides mSWI/SNF complexes with various capabilities including ATP hydrolysis, maintenance of structural integrity, and binding of DNA and histones. Mutations in *SMARCB1* (BAF47) represented the first link between SWI/SNF complexes and cancer, upon the discovery by Delattre and co-workers that 98% of malignant rhabdoid tumors exhibit biallelic inactivation of *SMARCB1* [47]. Exome sequencing studies have further

revealed that members of these complexes are mutated in over 20% of cancers and that certain subunits are recurrently mutated in specific cancer types [48,49]. For example, *ARID1A* (BAF250A) is mutated in 46% of ovarian clear cell carcinoma cases; *SMARCA4* (BRG1) is mutated in 10–35% of non-small cell lung cancer cases; and *PBRM1* (BAF180) is mutated in 41% of clear cell renal cell carcinoma cases [50–53]. These findings underscore that each subunit has a specific role as a member of these complexes and that loss of specific subunit functions results in oncogenic transformation only in the context of certain cellular lineages.

## Composition and function of PcG complexes

Genes that produce the PcG proteins were first discovered in *Drosophila* as repressors of *Hox* gene expression in development and differentiation [20]. Homozygous loss of PcG gene members resulted in dramatic homeotic transformation in the body segmentation of flies [54–58]. Protein purifications from *Drosophila* revealed that individual PcG members formed a protein complex, called polycomb repressive complex 1 (PRC1), which was capable of compacting nucleosomes [23]. Further purifications revealed a second complex with distinct members, called polycomb repressive complex 2 (PRC2), possessing a methyltransferase specific for lysine 27 on the H3 tail (H3K27) [59,60]. Homologous PRC1 and PRC2 complexes exist in mammals and similar to SWI/SNF complexes, subunits of each complex have multiple paralogs to facilitate greater compositional and thus functional diversity for their wide-ranging repressive roles in both embryonic and tissue development [61]. Canonical PRC1 complexes (cPRC1) consist of CBX, PHC, PCGF, RING, and SCMH proteins, with non-canonical PRC1 complexes (ncPRC1) replacing CBX with RYBP or YAF2 along with a number of additional proteins specific to different ncPRC1 forms (Figure 1B) [22]. The primary chromatin modifying role of PRC1 comes from the RING subunit, which is an E3 ligase which catalyzes the ubiquitination of lysine 119 of histone H2A (H2AK119). The core members of PRC2 complexes are SUZ12, EED, RBBP4/7, and either EZH1 or EZH2, with a variety of accessory proteins involved in chromatin binding (Figure 1C). The EZH1/2 proteins are SET methyltransferases that produce mono-, di-, and tri-methylated H3K27 in a SAM-dependent manner. The core member EED binds H3K27me3 to further propagate methylation of H3K27me3 by PRC2, and it has been shown that the methyltransferase activity of EZH1/2 requires a minimal complex that includes SUZ12 and EED [62–64]. While the potential role of cooperation between PRC1 and PRC2 complexes in transcriptional repression is still being fully elucidated, these two complexes modify chromatin through the placement of the H2AK119 ubiquitin and H3K27me3 marks to compact chromatin and inhibit transcription.

## Gain-of-function oncogenic mechanisms of mSWI/SNF complexes within sarcomas

### Synovial sarcoma: the gained SS18–SSX subunit of mSWI/SNF complexes results in loss of PRC2-induced repression

Synovial sarcoma comprises 8–10% of soft-tissue malignancies and forms solid tumors that in over 80% of cases arise in the extremities [65,66]. Synovial sarcoma occurs in both adult

and pediatric patients, with a median age of onset within the third decade of life [67]. Tumors are high-grade and prone to metastasize to the lungs, with a higher frequency of metastasis observed in adult patients [68]. Synovial sarcomas are divided into three histological subtypes consisting of monophasic (spindle cells only), biphasic (both spindle and epithelial cells that form glandular patterns), and a third class termed poorly differentiated synovial sarcoma (PDSS) characterized by an undifferentiated/round cell appearance [69]. The hallmark genetic event, initially identified in 1994 and present in nearly 100% of cases, is a recurrent chromosomal translocation, t(X;18)(p11.2;q11.2), that fuses an *SSX* gene family member (*SSX1*, *SSX2*, and rarely *SSX4*) to the *SS18* gene [1,70,71]. The resulting product is the SS18–SSX fusion protein, in which the last eight C-terminal amino acids of SS18 are replaced by 78 C-terminal amino acids of SSX. Clinical diagnosis for this disease is confirmed through detection of *SS18–SSX* by next-generation sequencing (NGS), RT-PCR or FISH [72–74]. The *SSX* gene family consists of nine members with high (73–92%) homology that are restricted in gene expression to the testes [75]. The *SSX* fusion partner is *SSX1* in two-thirds of cases and *SSX2* in the remaining third of cases, with a few documented cases of *SSX4* [70,76,77]. As the *SS18* gene is ubiquitously expressed, following translocation, the resulting SS18–SSX fusion protein is expressed in all tumor cells. The presence of this fusion is the hallmark genetic feature of these tumors and as there are no other recurrent cytogenetic abnormalities, it has been determined that expression of this fusion is necessary and sufficient for the initiation and progression of synovial sarcoma [78,79].

Historically, genes encoding mSWI/SNF complex members have been considered as tumor suppressors, in that protein-level loss of a subunit member results in oncogenesis in a particular cellular context [80]. Synovial sarcoma presents an example in which mSWI/SNF complexes gain oncogenic activity through aberrant fusion of the *SS18* subunit gene to *SSX* to form the *SS18–SSX* oncogene. The SS18–SSX fusion protein is stably and dominantly incorporated into BAF complexes, replacing wild-type SS18, and furthermore, results in eviction of the BAF47 (SMARCB1/SNF5/INI1) subunit, a known tumor suppressor [81] (Figure 2A). Consequently, upon expression of SS18–SSX, the majority (>90%) of BAF complexes become altered to form a population of oncogenic SS18–SSX-containing complexes. The eviction of BAF47 from these complexes results in its proteasome-mediated degradation resulting in decreased BAF47 protein levels in these tumors, as determined by immunohistochemical staining of paraffin-embedded primary tumor samples [81–84]. This gain of SS18–SSX and loss of BAF47 represent co-occurring alterations to BAF complexes; their respective contributions to the oncogenic mechanism in this malignancy have not, to date, been elucidated. SS18–SSX has been demonstrated to provide BAF complexes with novel targeting capabilities on chromatin to activate aberrant transcription. For example, SS18–SSX-containing BAF complexes localize to the *SOX2* locus, at which levels of the PRC2-placed H3K27me3 repressive mark are then reduced, resulting in expression of the *SOX2* mRNA transcript [81]. This exemplifies aberrantly gained genomic targeting of BAF complexes to a site of PRC2 repression. While the cell of origin of synovial sarcoma is unknown, there likely exists a highly specific cellular context that is permissive of SS18–SSX-mediated BAF complex recruitment and gene activation that leads to oncogenic transformation, as expression of SS18–SSX in most primary fibroblast cultures (as well as

other cell lines) does not yield transformation. Some studies have suggested that the cell of origin of these tumors may be of a myogenic lineage; however, the specific features and/or stage of myogenic differentiation remain incompletely defined [85].

### **Ewing sarcoma: gained functionality of mSWI/SNF complexes through interaction with the FET family protein EWSR1**

Ewing sarcoma represents the second most common pediatric bone cancer, with tumors that arise in bones or surrounding soft tissues [86]. Tumors most often present in teenagers and young adults, and these cancers, similar to cases of synovial sarcoma, share a pathognomonic chromosomal translocation [86]. In 90% of cases, this translocation results in the *EWSR1* gene becoming fused to the *FLI1* gene, which encodes for an E-twenty-six (ETS) transcription factor [3]. Less common but related translocations in the remaining 10% of Ewing sarcoma cases form *EWSR1* gene fusions with other members of the ETS family of transcription factors, including *ERG*, *ETV1*, *E1AF*, and *FEV* [87–90]. Recent exome sequencing efforts indicate that these resulting oncogenic gene fusions are the only reoccurring genetic alterations in this malignancy as few other mutations are detected [91]. Studies have demonstrated that expression of the fusion protein is sufficient for transformation of mesenchymal stem cells [92–97].

While exome sequencing studies have identified extensive mutations in dedicated subunits of mSWI/SNF complexes, the contribution of mSWI/SNF complexes in oncogenesis likely extends well beyond perturbation to mSWI/SNF complex members themselves [49]. Indeed, mSWI/SNF complex mistargeting due to genetic alterations to transient protein interaction partners, such as transcription factors, represents one route to altered, cancer-specific gene activation. Ewing sarcoma is an example of such a malignancy where mSWI/SNF complexes acquire gained oncogenic function due to genetic perturbation to a protein interaction partner. The ETS DNA binding domain of FLI1 enables the EWS–FLI1 fusion to aberrantly bind DNA at GGAA repeats [3,98–101]. However, binding of FLI1 alone at GGAA repeats is not sufficient for inducing oncogenic gene activation and thus this oncogenic fusion possesses a gained function [102]. Mass spectrometry-based proteomics coupled with biochemical validation experiments demonstrate that the EWSR1 protein binds BAF complexes and that the EWS–FLI1 fusion retains this binding capability [102]. This interaction between EWS–FLI1 fusion and BAF complexes represents a weak, transient interaction, in comparison to the highly stable interactions of integrated BAF complex subunits. Importantly, the prion-like domain of the EWSR1 N-terminus confers unique phase transition properties, providing BAF complexes with gain-of-function capability to be recruited to GGAA repeats and to remodel closed chromatin for enhancer activation (gained H3K27ac levels) that leads to oncogenic transcription [102] (Figure 2B). This is a novel example of a perturbation to mSWI/SNF complexes that drives cancer resulting from a gained interaction with an oncogenic fusion protein in the absence of any genetic perturbations to individual subunit members. It highlights that identifying malignancies driven fully or in part by mSWI/SNF complexes will not be comprehensively flagged through exome sequencing studies, but instead requires rigorous biochemical interrogation into both alterations in complex composition and transient interactions with transcription factors or other protein partners.



## Oncogenic loss-of-function mechanisms of mSWI/SNF and PcG complexes in sarcomas

### ***SMARCB1*-deficient sarcomas: loss of mSWI/SNF complex function results in gain of PRC2-induced repression**

Biallelic inactivation of *SMARCB1* (BAF47/hSNF5/ INI1) is the defining genetic alteration in a specific set of malignancies. Protein loss of BAF47 occurs in ~98% of malignant rhabdoid tumor (MRT) and atypical teratoid/rhabdoid tumor (AT/RT) cases, and over 90% of epithelioid sarcoma (EpS) cases [47,103–105]. These sarcomas are all aggressive tumors arising in the kidney and soft tissues (MRT) and central nervous system (AT/RT) of pediatric patients in the infant and toddler age groups, and distal and proximal sites of extremities (EpS) in young adults. The genetic perturbation of BAF47 has also been documented and implicated in a number of other neoplasms including poorly differentiated chordoma, renal medullary carcinoma, and meningiomas and schwannomas in patients with schwannomatosis [106–109]. Germline mutation resulting in loss of a single allele of *SMARCB1* predisposes the development of MRT or another BAF47-deficient cancer owing to loss of *SMARCB1* via a second genetic event within certain cell types. These sarcomas possess stable genomes with low mutational burdens, suggesting that BAF47 loss is responsible for tumor initiation and progression [91,110]. BAF47 has been well established as a tumor suppressor as conditional biallelic inactivation of *Smarchb1* (Baf47) in a mouse model resulted in tumor formation in 100% of cases with a median onset of 11 weeks, representing the most rapid inception of tumorigenesis by deletion of a single gene currently documented [111].

BAF47 is a stable subunit in both BAF and PBAF assembly forms of mSWI/SNF complexes, and the discovery of BAF47 loss in MRT was the first evidence that alterations to the composition of mSWI/SNF complexes perturbed their tumor suppressive functions [47]. This is an example of a genetic event which results in loss of protein expression of a single subunit member, causing altered stability, targeting capability, or enzymatic activity of mSWI/SNF complexes. Studies examining the role of BAF47 in complex stability have been somewhat contradicting, with some studies suggesting that complexes lose the ability to assemble in the absence of BAF47, while others demonstrate that complexes lacking BAF47 form stable complexes that have diminished chromatin-binding capabilities [24,112–116]. Providing further support that BAF47 is not required for the structural integrity and assembly of mSWI/SNF complexes, a study in yeast examining SWI/SNF complexes lacking the Snf5 subunit determined that aberrant complexes were formed that exhibited dramatically attenuated ATPase function [117]. In the context of MRT, mSWI/SNF complexes lacking BAF47 demonstrated decreased affinity for binding chromatin, which resulted in significantly decreased genome-wide targeting [118]. Reintroduction of BAF47 expression and subsequent incorporation into BAF and PBAF complexes resulted in gained occupancy of BAF complexes on enhancers and PBAF complexes on gene promoters, leading to enhancer (increased H3K27ac levels) and gene activation [118] (Figure 3A). In the absence of recruitment by BAF complexes due to loss of BAF47 assembly, a number of bivalent promoters of neural and kidney development-associated genes are silenced owing to the co-presence of the activating H3K4me3 and repressive H3K27me3 marks, and thus are

transcriptionally repressed [118]. Reincorporation of BAF47 into mSWI/SNF complexes displaces PRC2 complexes at these bivalent genes, resulting in reduction of the H3K27me3 mark and resolution of these bivalent genes toward activation to permit cell development toward terminal differentiation [118]. The ability of mSWI/SNF complexes to oppose PcG occupancy and induce repression is reliant on the BAF47 subunit. This is supported by previous evidence that mSWI/SNF complexes lose the ability to oppose PcG complexes in the absence of BAF47 at the p16<sup>INK4A</sup> locus and now it is clear that this opposition is lost at a number of bivalent genes genome-wide [119,120].

### **Malignant peripheral nerve sheath tumors (MPNSTs): loss of PRC2 complex function results in transcriptional activation**

MPNSTs are highly aggressive, soft-tissue tumors that arise in the cellular components associated with peripheral nerves [121]. These tumors can arise in pediatric patients, but are more prominent in adult patients [121]. They have a high propensity to metastasize and are often resistant to the standard treatment of radiation and chemotherapy, and patients with this disease have a 5-year survival rate of 20% [122]. Cases of MPNSTs account for 5–10% of all soft-tissue sarcoma cases and are composed of three types: sporadic (45% of cases), associated with neurofibromatosis type 1 or NF1-associated (45% of cases), and associated with previous radiotherapy (10% of cases) [123–125]. NF1 is an autosomal-dominant hereditary cancer syndrome that affects 1 in 3000 individuals due to a germline mutation that inactivates one allele of the *NF1* gene [126]. Acquisition of a second somatic mutation that silences the *NF1* gene of the allele inherited from the unaffected parent is the key driver in developing neurofibroma, which forms benign tumors and increases the risk of developing the more aggressive MPNSTs [127]. Whole-exome sequencing studies and transcriptional analysis of MPNST cases of all three types have uncovered recurrent inactivating genetic alterations to *NF1* (72% of non-NF1-associated cases), *CDKN2A* (81% of all cases), and multiple subunit members of the PRC2 complex (70–92% of all cases) [128–130].

While MRT and synovial sarcomas discussed above contain genetic alterations to mSWI/SNF members that result in gained and lost H3K27me3-mediated repression, respectively, MPNST-associated mutations in PRC2 complex components directly cause loss of the H3K27me3 mark. The genetic alterations to PRC2 complex members consist of loss-of-function mutations to *EED* or *SUZ12* that result in decreased levels of the H3K27me3 mark detected by immunohistochemical staining of paraffin-embedded tumor samples from patients (Figure 3B) [128]. Decreased H3K27me3 levels in MPNSTs due to loss of function of PRC2 complexes are associated with poor survival [131]. PRC2 loss has not been observed in cases of neurofibroma alone, the precursor to a subset of MPNST cases. Recent transcriptional analyses in MPNSTs have revealed that this loss of PRC2 function results in increased expression of master regulator genes, known to be repressed by PRC2, as would be expected given the well-documented opposition between PRC2 and TrxG complexes [128]. However, to date, specific changes to the activity or localization of mSWI/SNF complexes have not been reported in this disease setting. As evidence that this loss of PRC2-mediated transcriptional repression contributes to tumorigenesis, reintroduction of *SUZ12* expression in a cell line established from MPNSTs with a *SUZ12* silencing mutation results in substantially decreased cell growth accompanied by decreased expression of



developmental genes, increased recruitment of PRC2 complexes to gene promoters, increased levels of H3K27me<sub>3</sub>, and subsequent loss of the H3K4me<sub>3</sub> and H3K27ac activation marks [128,130]. Cases of MPNSTs display clinical and histological diversity due to divergent differentiations, yet possess and share a convergent set of genetically perturbed molecular pathways (stemming from *NFI*, *CDKN2A*, and PRC2 complex gene mutations). The wide range of genes silenced by PRC2 complexes to prevent aberrant differentiation toward an alternative cell lineage suggests a mechanism by which perturbation of PRC2 complexes can result in disruption of gene repression, allowing for the formation of tumors through activation of one of a number of differentiation pathways. Due to the contribution of loss-of-function genetic alterations to PRC2 complexes in driving tumorigenesis, MPNST is a unique setting where genes encoding PRC2 complex members act as tumor suppressors rather than oncogenes [132–134].

## Therapeutic strategies and conclusions

The discovery of actionable synthetic lethal dependencies resulting from an imbalance of mSWI/SNF and PcG complex activities in sarcomas presents an important foundation for the identification of targeted therapies. Large, genome-scale fitness dropout screens using shRNA-mediated gene knockdown and CRISPR-Cas9-mediated gene knockout have been carried out across hundreds of cancer cell lines, including some derived from sarcomas [135–137]. These screens are designed to identify genes and gene pathways that are essential for proliferation selectively within the context of specific sarcoma subtypes and their underlying genetic abnormalities. Due to the diverse nature of mSWI/SNF–PcG opposition genome-wide in sarcomas and the resulting heterogeneity in oncogenic transcription, it remains to be determined whether there are unique downstream dependencies that are shared across any individual sarcoma subtype. Consequently, as the mechanistic underpinnings of epigenetic dysregulation are further uncovered, altered mSWI/SNF or PcG activity driving oncogenic transformation may be targeted directly.

A range of small molecules that inhibit PRC2-mediated deposition of H3K27me<sub>3</sub> by targeting the EZH2 and EED subunits have been reported, and are actively being evaluated across a range of malignant contexts [138–140]. Depletion of EZH2 protein levels has been shown to induce apoptosis in some MRT cell lines, which is consistent with the observed gain of PRC2-induced repression in BAF47-deficient sarcomas [119]. Potent and specific EZH2 inhibition has been shown to attenuate BAF47-deficient solid tumor growth in preclinical models [141,142]. A phase 2 clinical trial () investigated the anti-tumor activity of EZH2 inhibition in BAF47-deficient tumors and reported potentially promising anti-tumor response within the patient cohort [143]. This trial included synovial sarcoma patients due to the low, residual levels of BAF47 protein expression present in these tumors, yet results to date indicate a lack of objective response in synovial sarcoma patients following treatment with EZH2 inhibitor [144]. This is likely due to the fact that BAF47 loss in this disease is a concurrent event along with gain of the SS18–SSX fusion. Rather than loss of BAF47 resulting in gain of PRC2-mediated repression, as is the case in other BAF47-deficient sarcomas, the SS18–SSX-containing BAF complexes appear to lead to gene activation at loci such as the *SOX2* locus. While further studies are ongoing, this provides preliminary evidence that oncogenesis is dependent on gained mSWI/SNF complex-

mediated transcription rather than overactivity of PRC2-induced gene repression. Consequently, targeted therapies for synovial sarcoma patients will likely need to target mSWI/SNF complex activity or other regulators required for their activity, as opposed to PRC2 function. Furthermore, while it is clear that loss of PRC2 function in MPNSTs results in gene activation, it remains to be determined whether a transcriptional activator, such as a SWI/SNF family chromatin remodeler, contributes to this activation. The characterization of such an activator and validation of its essentiality in cell proliferation of MPNSTs would suggest a promising therapeutic route in this disease.

Reversing the oncogenic activity of fusion proteins could be achieved through chemical biology-centered strategies that inhibit a certain function of the protein or promote protein-specific degradation. The SS18–SSX fusion results in novel mSWI/SNF targeting which suggests that this fusion interacts directly and uniquely with chromatin or a chromatin-associated protein [81]. Identification of a specific interaction partner would provide the foundational knowledge required for the design of small molecule screens to discover chemical ligands capable of disrupting SS18–SSX chromatin-targeting activity. Similarly, the EWS–FLI1 fusion interacts with mSWI/SNF complexes to activate enhancers through the EWSR1 member of the fusion protein [102]. Discovery of a chemical ligand that inhibits this interaction with mSWI/SNF complexes could be used to prevent the mistargeting of complexes driving this disease. Modern chemical biology techniques have laid the groundwork for using a small molecule chemical binder of a protein as a targeting module for protein-specific proteasome degradation. The selective chemical binder is tethered to a module that recruits an E3 ubiquitin ligase, rendering the targeted protein marked for degradation [145–148]. Discovery of a high-affinity, specific chemical binder to either the SS18–SSX or the EWS–FLI1 fusion could be used to target these fusions for protein degradation using this approach. While these binders do not currently exist and would require a great deal of biochemical and preclinical validation prior to their use in a clinical setting, this is a promising strategy for the protein-level removal of oncogenic fusions in sarcomas.

In summary, there remains a major need to mechanistically understand the functional ramifications of recurrent genetic abnormalities that drive sarcomagenesis via gene regulatory changes, specifically due to alteration to chromatin regulatory complexes. This knowledge will be requisite for the design and development of cancer-specific therapies tailored to address the underlying molecular events driving tumorigenesis within each sarcoma subtype.

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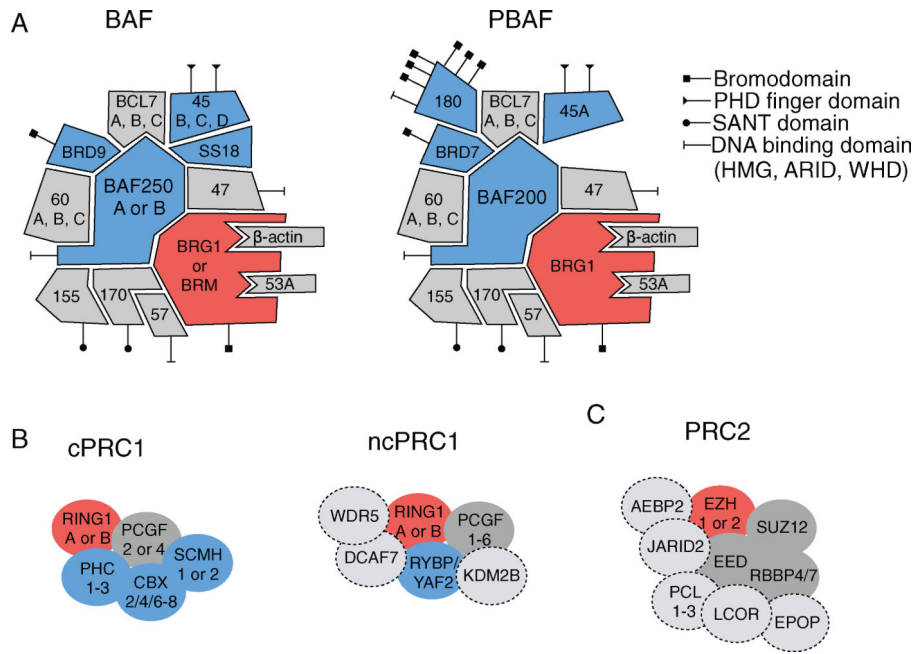
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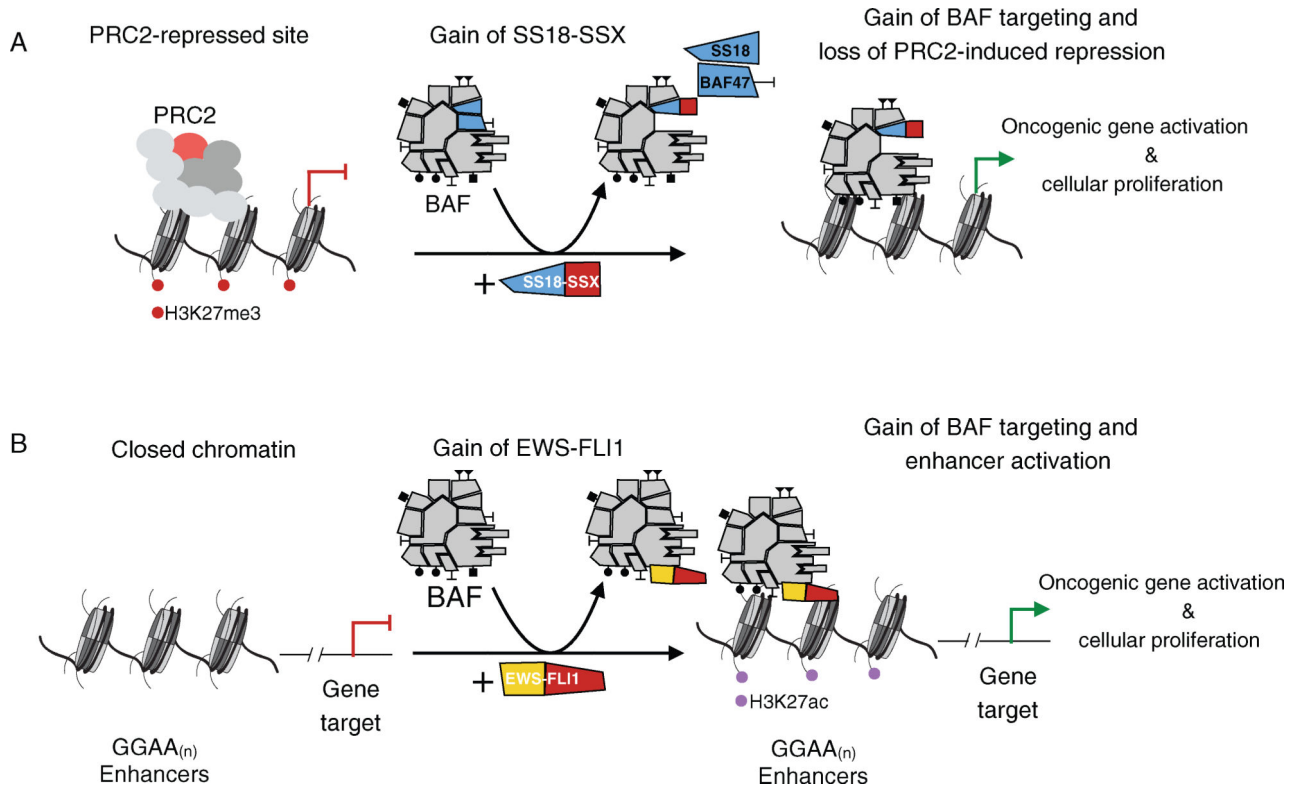
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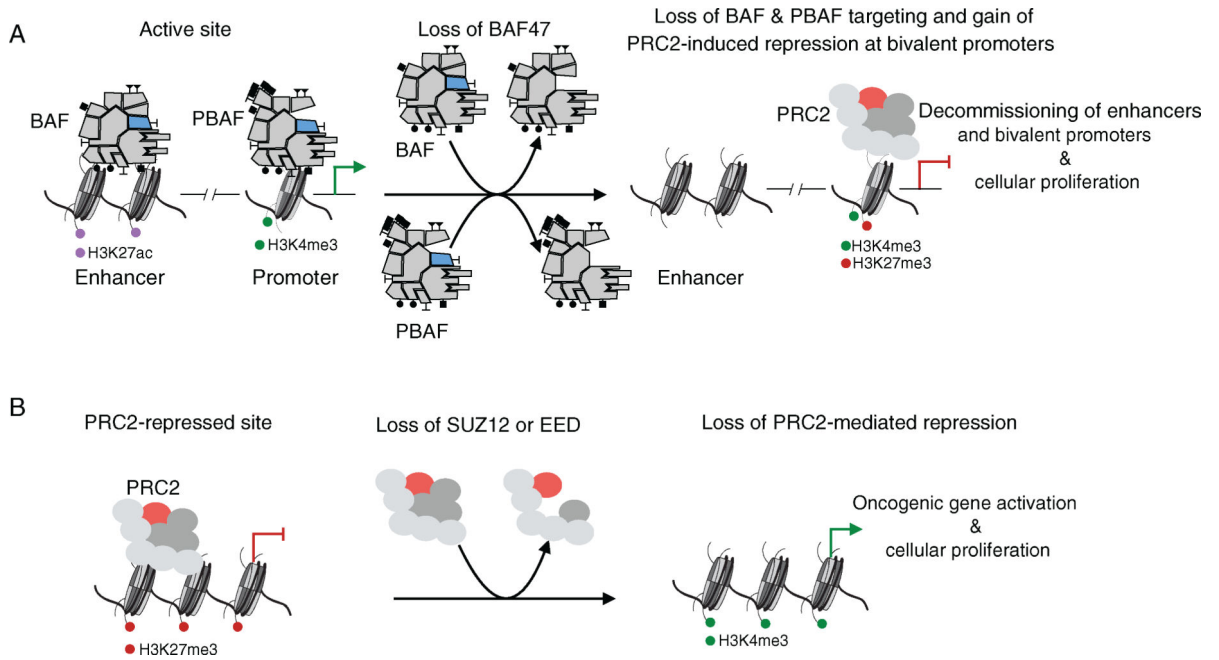
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**Figure 1.** Composition of mammalian SWI/SNF and polycomb group complexes PRC1 and PRC2. (A) mSWI/SNF chromatin remodeling complexes assemble into two distinct complexes, each containing an ATPase subunit (depicted in red), subunits specific to one of the two assembly forms (depicted in blue), and core subunits present in both complex forms (gray). DNA-binding and histone recognition domains are marked according to the legend. (B) Polycomb repressive complex 1 assembles into canonical PRC1 (cPRC1) and non-canonical PRC1 (ncPRC1) complexes possessing a ubiquitination subunit (depicted in red), subunits unique to one complex form (depicted in blue), and accessory subunits that are incorporated into different ncPRC1 forms (depicted by a dashed line). (C) Polycomb repressive complex 2 (PRC2) assembly includes a methyltransferase subunit (depicted in red) which places the H3K27me3 repressive mark, other core subunits (shown in gray), and accessory subunits that are incorporated into different PRC2 forms (depicted by a dashed line).



**Figure 2.** Fusion oncoprotein-bound mSWI/SNF complexes activate oncogenic transcription via cancer-specific genomic targeting. (A) In synovial sarcoma, the SS18–SSX fusion is incorporated into BAF complexes, resulting in the eviction of wild-type SS18 and BAF47 subunits. These SS18–SSX-containing complexes are targeted to sites of PRC2-mediated repression, such as *SOX2*, leading to a reduction in H3K27me3 levels and activation of transcription. (B) In Ewing sarcoma, the EWS–FLI1 fusion transiently interacts with BAF complexes and results in cancer-specific targeting to GGAA microsatellites. This *de novo* targeting of BAF complexes leads to increased H3K27ac levels, enhancer activation, and oncogenic transcription.



**Figure 3.** Genome-wide loss of mSWI/SNF and PRC2 complex activity and targeting results in oncogenic transcription. (A) In BAF47-deficient sarcomas, such as malignant rhabdoid tumor and epithelioid sarcoma, the loss of BAF47 incorporation into BAF and PBAF complexes results in residual complexes with decreased chromatin affinity. BAF complex occupancy and H3K27ac levels are reduced genome-wide at enhancers and PBAF complexes are attenuated at gene promoters, while PRC2 occupancy increases at active gene promoters, leading to deposition of H3K27me3 and the creation of transcriptionally-silent bivalent gene promoters. (B) In cases of malignant peripheral nerve sheath tumors with SUZ12 or EED subunit loss, PRC2 occupancy and H3K27me3 levels are reduced, contributing to increased activation of oncogenic transcription.



**Table 1.**

An overview of the chromatin regulator-associated genetic abnormalities in sarcomas

Sarcoma subtype	Gene (protein) involved	Associated genetic abnormality (% of cases)	References
Synovial sarcoma (SS)	<i>SS18, SSX1/2/4</i>	Chromosomal translocation forming <i>SS18-SSX1/2/4</i> fusion (~100%)	[1,2,70]
Ewing sarcoma (ES)	<i>EWSR1, FLI1, ERG, ETV1, E/AF, FEV</i>	Chromosomal translocation forming <i>EWS-FLI1/ERG/ETV1/E/AF/FEV</i> fusion (~100%)	[3,87-90]
Malignant rhabdoid tumor (MRT)/atypical teratoid/rhabdoid tumor (AT/RT)/epithelioid sarcoma (EpS)	<i>SMARCB1 (BAF47)</i>	Homozygous loss (98%, 98%, > 90%)	[47,103-105]
Malignant peripheral nerve sheath tumors (MPNSTs)	<i>SUZ12, EED</i>	Heterozygous or homozygous loss (70-92%)	[128-130]