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Chromatin regulatory mechanisms and therapeutic opportunities in cancer

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Abstract

Research over the past several decades has unmasked a major contribution of disrupted chromatin regulatory processes to human disease, particularly cancer. Advances in genome-wide technologies have highlighted frequent mutations in genes encoding chromatin-associated proteins, identified unexpected synthetic lethal opportunities and enabled increasingly comprehensive structural and functional dissection. Here, we review recent progress in our understanding of oncogenic mechanisms at each level of chromatin organization and regulation, and discuss new strategies towards therapeutic intervention.

The dynamic control of genomic architecture is required for virtually every cellular function. Nearly 2 m of DNA is organized in each cell nucleus by interacting with histones to form chromatin, a structure that enables its packaging into a less- than 10-μm diameter space[1]. Chromatin can be regulated by several processes, including modifications of DNA[2], modifications of histones[3] and protein complexes that remodel its architecture[4]. These mechanisms function individually and in concert to modulate genome-wide topology and gene expression, thereby regulating cell differentiation, cell division and tissue and organismic development.

Disruptions in chromatin regulation can have profoundly detrimental effects. The role for chromatin regulatory processes in development and disease has been studied in depth and has recently been brought to the forefront of attention by exome-wide and genome-wide studies, which have identified mutations in genes involved in chromatin organization and regulation in over 50% of cancers[5–7]. In a subset of cancers, such mutations represent the

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Competing interests

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sole genetic abnormalities, providing strong support for their initiating, causative functions, rather than roles as permissive passenger mutations.

The impetus to mechanistically understand chromatin regulatory machinery in diseases such as cancer stems from several important features, perhaps the most compelling of which is the fact that epigenetic changes are, in principle, reversible. However, considering the diverse functions of each class of chromatin regulators in normal tissues and disease contexts, safe and efficacious therapeutic targeting remains a major yet promising challenge. In this Review, we discuss the state of the field investigating epigenetic dysregulation in cancer and highlight the range of current and emerging opportunities for clinical development.

Mechanisms governing chromatin structure

The primary functional unit of chromatin is the nucleosome core particle, which consists of ~146 base pairs of DNA wrapped around a histone octamer assembled by two molecules of histones H2A, H2B, H3 and H4 (ref. [8]) (Fig. 1a). Linker DNA connects nucleosome core particles to create the classical ‘beads on string’ analogy that is commonly used to describe primary chromatin structure. The linker histone protein H1 binds to the nucleosome core particle at the DNA entry and exit sites to impart nucleosome stability and facilitate higher-order chromatin structure[9,10] (Fig. 1a). Chromatin can be either densely packed, in the form of heterochromatin, which is largely inaccessible to transcriptional machinery and hence encompasses inactive genes, or as open and accessible euchromatin, which contains greater numbers of active genes. The dynamic, tightly controlled regulation of these chromatin configurations is essential for timely, coordinated and appropriately scaled gene expression.

DNA- and histone-modifying proteins, and ATP-dependent chromatin remodelling complexes (CRCs) are the three groups of proteins that facilitate changes in chromatin topology and regulation. DNA-modifying proteins place covalent modifications, such as cytosine methylation (5-methylcytosine (5mC) or 5-hydroxymethylcytosine (5hmC)), on DNA itself[2,11] (Fig. 1b), rendering genes controlled by such sequences as inactive or active, respectively. Histone-modifying proteins mediate >200 distinct covalent post-translational modifications on histone globular domains or on histone tails to alter local chromatin compaction, nucleosome dynamics, recruitment of other chromatin-bound proteins, and hence, transcription[3,12] (Fig. 1c). Finally, a diverse group of CRCs comprising > 100 different protein subunits utilize ATP hydrolysis to mobilize nucleosomes, thereby modulating chromatin structure and regulation[4] (Fig. 1d). CRC activity is believed to locally increase DNA accessibility through nucleosome sliding or ejection. However, recent studies also suggest ATPase-dependent functions that affect the targeting and activity of other chromatin regulatory proteins[13,14]. Taken together, genomic architecture and gene expression are governed by a diverse collection of proteins and modifications, acting both globally and focally at specific sites to orchestrate dynamic processes, such as cell division, differentiation and development, as well as basal maintenance of cell homeostasis.

History and mechanisms of covalent DNA modifications

DNA methylation regulates gene silencing including X-chromosome inactivation, genomic imprinting and tissue-specific transcriptional repression[15,16]. In vertebrates, 5mC is predominantly found in CpG dinucleotides and localizes to transcriptionally silenced centromeres, telomeres and repetitive transposable elements (short interspersed nuclear elements and long interspersed nuclear elements). Additionally, regions with highly methylated CpG density have been shown to be associated with high nucleosome occupancy in facultative and constitutive heterochromatin[17]. 5mC is present throughout the vertebrate genome with distinct localizations required for cell differentiation during development and in somatic cells[2]. Although most CpG dinucleotides are methylated, dense regions called CpG islands, which overlap with over half of mammalian promoters, are largely unmethylated and therefore active. Early studies have established global levels of hypomethylation with focal hypermethylation of promoters and enhancers as a common feature among several cancers[18] (Fig. 2a).

DNA methyltransferases

DNA methylation is catalysed by DNA methyltransferases (DNMTs), which deposit methyl groups on the carbon-5 position of cytosine via the *S*-adenosyl-methionine methyl donor[19] (Fig. 2b). Among the five members of the DNMT family, DNMT3A and DNMT3B are canonically considered de novo methyltransferases that localize to pericentromeric heterochromatin to silence gene expression[20,21]. DNMT1 serves more general maintenance-centred roles owing to relaxed substrate specificity and preference for methylation of hemimethylated CpG dinucleotides, particularly in proliferating cells[22]. Collectively, DNMTs are involved in appropriate haematopoietic stem cell differentiation, tissue development, adult tissue integrity[23] and immune function[24].

DNA methylation patterns are disrupted in various malignancies[25–30], but aberrations in the genes encoding DNA methylation and demethylation machinery have only been recently identified. *DNMT3A* mutations, including those found in the hotspot catalytic domain[31], are present in ~25% of adult acute myeloid leukaemia (AML) cases[32–34], pointing to DNMT3A as an important tumour suppressor[31] (Fig. 2b).

Although genome-wide distributions of DNMTs, particularly those of DNMT3A/B, have been extensively studied[35,36], the mechanisms responsible for their chromatin deposition and activity in steady state or in cancer have not been fully elucidated. The crystal structure of the DNMT3A–DNMT3L–DNA complex has revealed the mechanisms governing DNMT3 substrate recognition and enzymatic specificity[37], by demonstrating that DNMT3A monomers attack two CpGs through a target recognition domain. Intriguingly, DNMT3A cancer-associated somatic missense mutations of the substrate-binding domain decrease in vitro methyltransferase activity, thereby inducing CpG hypomethylation[37]. Similar studies of DNMT1 indicate that histone post-translational modifications recruit and activate DNMT1 at specific DNA methylation sites[38]. Cytidine analogues, such as 5-azacytidine and 5-aza-2′-deoxycytidine (also known as decitabine) are potent DNMT inhibitors that have shown modest efficacy in the treatment of AML, chronic myelomonocytic leukaemia and

myelodysplastic syndromes[39], and are potentiated in combination with other epigenetic and/or chemotherapeutic agents[40].

TET enzymes

The identification and biochemical characterization of ten-eleven translocation (TET) enzymes over the past decade has been a particularly important advance in the field of epigenetics. The fact that ~7–10% of all patients with AML harbour deletion or truncating mutations in *TET* genes highlights the importance of characterizing bidirectional implications of epigenetic modifications in cancer. The TET1–3 enzymes directly oppose the activity of DNMTs by erasing DNA methylation through the iterative oxidation of 5mC to both relatively stable (5hmC) and transient (5-formyl-methylcytosine (5fC) and 5-carboxyl-methylcytosine (5caC)) derivatives, in an Fe(II) and α-ketoglutarate-dependent mechanism[41] (Fig. 2b). The 5fC and 5caC derivatives are then thought to be processed by thymine DNA glycolase and DNA base excision repair machinery[11,42], resulting in unmethylated cytosines. Structural investigations suggest that catalytic domains of TET1–3 preferentially bind to CpG dinucleotides[43]. TET1 and TET3 each harbour an additional CXXC domain, which favours binding of 5mC-, 5hmC- and 5caC-modified CpGs[44,45]. Excitingly, recent results using novel TET inhibitors in AML cell lines and mouse models suggest potential therapeutic benefits[46]. As this class of inhibitors advances towards the clinic, the development of readily available, paired genomics-based diagnostic approaches to determine *DNMT* versus *TET* mutational status in patient tumours will be required.

Histone modifiers and their implications in cancer

A large collection of histone tail modifications and the proteins that control them represent critical components of the chromatin regulatory system as they contribute to the positioning and function of chromatin regulatory proteins and protein complexes genome wide[47–49]. Histone-modifying enzymes are grouped into histone deacetylases (HDACs) and histone acetyltransferases, which control lysine acetylation; methyl transferases and demethylases, which regulate lysine methylation; arginine methyltransferases, which facilitate arginine methylation; and various kinases and phosphatases (Fig. 3a). Additional histone modifications include citrullination, SUMOylation, ADP ribosylation, deamination and crotonylation (reviewed elsewhere[12,49]). Over 150 histone-modifying proteins have been identified and their dysregulation can result in the inappropriate activation of oncogenes or, conversely, the inactivation of tumour suppressors[50–52].

Changes to global histone modification signatures are common in cancer (reviewed elsewhere[12,53,54]), therapeutic interventions for which have recently been reviewed[55]. Here, we highlight key examples of perturbed histone modification machinery in cancer, which have catalysed the development of several new targeted therapies. In particular, we examine HDACs, Polycomb group (PcG) repressive complexes 1 and 2 (PRC1 and PRC2), and mixed-lineage leukaemia (MLL; also known as KMT2A).

HDACs

HDACs and their associated deacetylation functions play important roles in cell-cycle regulation, apoptosis, DNA-damage repair and other cellular processes[56], and their dysregulation is commonly observed in cancer. For example, upregulation of classes I, II and IV HDACs is observed in breast and colorectal cancers, as well as haematological malignancies, whereas their downregulation is observed at lower frequencies[57]. Oncogenic fusions also deregulate HDAC activity, such as the AML–ETO (eight-twenty-one oncoprotein) and promyelocytic leukaemia protein–retinoic acid receptor-α fusions, which aberrantly retarget HDACs to repress AML and retinoic acid receptor-α target genes, respectively, resulting in cellular transformation[58–60]. Several HDAC inhibitors have been US FDA approved for cancer treatment or are currently being evaluated in clinical trials for both solid and haematological malignancies[61]. Given that HDAC inhibitor monotherapy has been largely ineffective in solid tumours[62], careful evaluation of various combination regimens is currently ongoing in clinical trials. As with other chromatin regulators, effective therapeutic responses may necessitate the development of potent small molecules that target specific rather than global HDAC activities.

PRC2 and EZH2

PcG proteins form multiprotein complexes that bind to chromatin and repress transcription through methylation and ubiquitination of histones. PRC2 catalyses the monomethylation, dimethylation and trimethylation of histone H3 (that is, H3K27me, H3K27me2 and H3K27me3, respectively) and is canonically associated with long-term transcriptional silencing through deposition of the H3K27me3 mark[63,64]. Enhancer of zeste homologues 1 and 2 (EZH1/2) are the mutually exclusive catalytic subunits of PRC2, which function in a multiprotein complex with EED, SUZ12 and additional subunits[65]. EZH2 has garnered substantial attention as both an oncogene and a tumour suppressor, even in the same cancer type (reviewed elsewhere[66]). Given that Polycomb can repress both oncogenes[67] and tumour suppressors[68,69], misregulation of either gene class could promote oncogenesis in a context-specific manner. Loss-of-function mutations of genes encoding PRC2 subunits have been identified in leukaemia, myeloid disorders and malignant peripheral nerve sheath tumours[70–73]. Conversely, EZH2 upregulation has also been implicated in various cancers, including melanoma and breast cancers[74], and gain-of-function mutations in the SET domain of EZH2 have been identified in diffuse large B cell lymphoma[75–77].

The EED subunit of PRC2 forms an aromatic cage around the H3K27me3 (or Jumonji/ARID domain-containing protein 2 (JARID2)-K116me3) mark, which allosterically activates EZH2. This is reliant on the ordered activity between the SRM and SET domains of EZH2 (refs. [78–80]). Mutations in this recognition site are implicated in cancer and Weaver's syndrome[81] and have been shown to impart deficient allosteric activation profiles. Remarkably, although mutants were deficient in activating genome-wide H2K27me3 deposition, no significant changes were observed in their genomic localization profiles[81], and allosteric activation of the hyperactive Y646N mutation present in diffuse large B cell lymphoma could be selectively inhibited. Such studies decouple chromatin binding and activity and suggest that allosteric inhibition of hyperactive EZH2 is a potential therapeutic avenue, consistent with newly developed allosteric inhibitors of PRC2, which target the EED

subunit[78,82,83]. These results indicate that decoupling chromatin binding, enzymatic activity and allosteric modulation of other non-enzymatic subunits may afford additional opportunities for complex targeting, for PRC2 as well as a concept for other chromatin regulatory protein complexes. EZH2 inhibitors show acceptable safety profiles and some efficacy in treating various cancers, including multiple myeloma[84], B cell non-Hodgkin's lymphoma and epithelioid sarcoma[85].

PRC1 and BMI1

PRC1 complexes contain a RING1 E3 ubiquitin ligase (RING1A/B), which catalyses the monoubiquitylation of histone H2A (that is, H2AK119Ub) and PcG RING finger proteins (PCGF1–6), which dictates downstream PRC1 subunit associations and thereby differential genome-wide localization of complexes[86]. Additional subcomplex-specific subunits include chromobox proteins, which bind to methylated histones, including PRC2-deposited H3K27 methylation, to promote gene silencing[87,88]. BMI1 (also known as PCGF4) is a PRC1 complex member that can form homodimers and heterodimers with RING1 (ref. [89]) or PHC subunits[90] that are important for chromatin compaction[91]. BMI1 is frequently upregulated in in AML[33,34,92] and it is necessary for selfrenewal and maintenance of healthy and leukaemic stem cells[93–96]. Depletion of BMI1 reduces proliferation and results in apoptosis of epithelial[97] and leukaemic cell lines, and in murine colorectal cancer xenograft models[98].

Therapeutic PRC1 targeting has only recently transitioned into preclinical and clinical settings. The first BMI1 inhibitor, PTC209 (ref. [98]), results in dose-dependent decreases of BMI1 protein levels, associated with global decreases of H2AK119Ub levels. Despite consistent anticancer activities in cell[99,100], murine xenograft[98,101] and preclinical[102] studies, the poor pharmacokinetic properties of PTC209 have stifled progress towards clinical trials[92]. The development of a more potent, orally available BMI1 inhibitor, PTC596 (ref. [103]), which also downregulates the anti-apoptotic factor MCL1, has progressed through phase I clinical trials for patients with advanced solid tumours[104] (ClinicalTrials.gov identifier:), and an additional trial for patients with ovarian cancer is ongoing (ClinicalTrials.gov identifier:).

MLL

The *MLL* gene encodes a histone methyltransferase, translocations of which are implicated in AML and acute lymphoid leukaemia with poor prognosis. MLL maintains the expression of *HOX* genes during development[105], and thus, its rearrangement often upregulates this gene cluster[106,107], including *HOXA9*, which is necessary for leukaemic cell proliferation[108]. Over 100 translocations and 60 fusion partners have been documented, with over two-thirds of these fusions involving members of the AEP (AF4–ENL (eleven nineteen leukaemia)–P-TEFb (positive transcription elongation factor b)) family[109–113]. Typically the amino-terminal MLL fusion breakpoint maintains CXXC, AT hooks and menininteracting regions, with loss of the carboxy-terminal SET, PHD and other domains[114]. The retained CXXC domain involved in CpG recognition is essential for transactivation and myeloid differentiation of MLL fusions[115]. Furthermore, the AEP subunit fusion partner recruits the DOT1-like protein (DOT1L) histone methyltransferase (Fig. 3b), resulting in aberrant H3K79 methylation at MLL target genes, such as *HOXA9* and *MEIS1*. A clustered regularly

interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9)-based screen in MLL–AF4 AML cells identified the *ENL* gene as a target of AML proliferation[116]. Targeted degradation further demonstrated that ENL regulates global transcription, with *HOXA10* and *MEIS1* exhibiting significant downregulation in expression.

Mutations targeting other histone-modifying methyltransferases also suggest new therapeutic potential. For instance, the DOT1L inhibitor pinometostat (also known as EPZ-5676) exhibited a favourable safety profile during phase I clinical trials, but requires further investigation, probably in combination with other therapies or in different cancer contexts, to maximally exploit its potential for efficacy[117] (ClinicalTrials.gov identifier:).

Oncohistones in cancer

Mutations in histone genes represent some of the most recently identified gene classes and have emerged through exome-wide sequencing of highly rare tumour types. Exome sequencing uncovered mutations in the genes encoding histone H3 variants (H3.1–H3.3) that convert lysine 27 to methionine (H3K27M) or glycine 34 to arginine or valine (H3G34R/V) in aggressive paediatric brain tumours (such as diffuse intrapontine glioma)[118,119] (Fig. 3c). These mutations specifically occur in the H3 tail, which prevents post-translational modification of H3 residues, and exhibit dominant functional changes. Moreover, specific mutations are found in tumours that arise in distinct brain regions, correlating with their distinct molecular characteristics[120]. Tumours harbouring the H3K27M mutation show dramatic reduction of H3K27me3 levels[121], suggesting that such mutants may dominantly inhibit normal lysine methylation pathways, such as those catalysed by PRC2 complexes[122]. Additional studies in *Drosophila* demonstrated that H3K27M mutant expression phenotypically mimics PRC2 loss[123,124] and mirrors the replacement of all histone H3s with a H3K27R mutant[125]. H3G34R/V mutations were also shown to block methylation of H3K36 in *cis* rather than inhibit bulk H3K27me3 or H3K36me3 in *trans*[113] (Fig. 3d). The precise, dominant mechanism(s) by which H3K27M affects the chromatin landscape and the activities of chromatin regulatory machineries remains to be identified. Some studies have suggested that H3K27M interacts with EZH2 (ref. [122]); however, others have found interaction with bromodomain-containing protein 4 (BRD4), consistent with increased histone acetylation levels observed in H3K27M mutant cells[126] (Fig. 3e).

Sequencing of chondroblastoma and giant cell tumours of the bone have identified additional oncohistone mutations[127]. Over 95% of chondroblastomas possess the histone H3.3 lysine 36 to methionine (H3.3K36M) mutation in the *H3F3B* gene, and about 92% of giant cell tumours of the bone harbour mutations of histone H3.3 glycine 34 to tryptophan or leucine (H3.3G34W/L)[127] (Fig. 3c). Similar to H3K27M, H3K36M reduces methylation of H3K36 by inhibiting the SETD2 and NSD2 methyltransferases[128,129] (Fig. 3e). These findings extend to mutations in genes encoding histone H1 in follicular lymphoma[130–132], which are predominantly single amino acid missense mutations scattered throughout the globular H1 domain involved in chromatin compaction, and have been shown to result in reduced association of histone H1 with chromatin[131] and DNMT3B[133].

Taken together, recurrent driver mutations in histone-encoding genes have added a new layer to the mechanisms of chromatin disruption in human cancer. Moreover, results such as those in paediatric brain tumours have underscored the specific cellular, developmental, anatomical and chromatin architecture contexts required for the high penetrance of such mutations.

Emerging mechanisms of ATP-dependent CRCs

Chromatin remodellers are multi-subunit complexes that use the energy of ATP hydrolysis to reposition, eject, slide or alter the composition of nucleosomes, enabling access of DNA-binding proteins and transcriptional machinery to DNA in order to facilitate gene expression[4] (Fig. 4a). Chromatin remodelling proteins play critical functions in cellular differentiation, division and DNA replication[4]. The four classes of chromatin remodellers include the mammalian SWI/SNF (mSWI/SNF (BAF)), imitation SWI, INO80, and nucleosome remodelling and deacetylation chromodomain helicase DNA-binding complexes[4] (Fig. 4b). Chromatin remodeller genes are evolutionarily conserved from yeast to humans, although higherorder organisms have evolved paralogous as well as new subunits in response to evolutionary pressures. The catalytic activity of each complex relies on the activity of a SWI/SNF2-like core ATPase/helicase, with accessory subunits harbouring DNA and histone-binding motifs. Across these families, combinatorial subunit assembly provides extensive complex diversity. Thus, most mammalian chromatin remodellers are typically further subclassified into multiple subcomplexes per family[134].

Among the CRC families, the most extensively mutated class is the mSWI/SNF complex (Fig. 4c). These complexes were originally characterized in yeast[135] in screens for mating-type switching and sucrose fermentation (hence the name SWI/SNF), and were later characterized in *Drosophila*[136] and mammals[137]. Over the course of evolution, SWI/SNF complexes have gained, lost and altered subunits to accommodate increasing genomic complexity and size. Recently, the modular organization and order of assembly of mSWI/SNF family complexes, including canonical BRG1/BRM-associated factor (BAF) complexes, polybromo-associated BAF (PBAF) complexes and newly discovered non-canonical BAF (ncBAF) complexes, were extensively characterized[138,139]. Exome-wide sequencing studies have revealed that > 20% of all cancers harbour mutations in mSWI/SNF-encoding genes[140,141], several of which are considered to be the key drivers of oncogenesis[142] (Fig. 4d). In particular, rare cancers, such as synovial sarcoma, malignant rhabdoid tumour (MRT), clear-cell meningioma and others, are known to be uniformly or near-uniformly caused by perturbations to mSWI/SNF complex subunit genes. In *Drosophila* and humans[13,143], SWI/SNF complexes oppose PRCs to activate gene expression[134] (Fig. 4e), suggesting that they may exert specific, ATP-dependent functions other than direct nucleosome remodelling. However, the biochemical and structural basis of such mechanisms remains unknown.

mSWI/SNF complexes were first linked to cancer through the identification of biallelic inactivation of the *SMARCB1* gene, which encodes the BAF47 subunit, in ~98% of MRT[144,145]. *SMARCB1* loss has been shown to result in decreased chromatin affinity, largely over distal enhancers, and an inability to oppose Polycomb-mediated repression at

bivalent promoters[146,147]. These data provide a mechanistic explanation for early cell-based and murine model-based findings, indicating that MRT may be uniquely sensitive to PRC2 inhibition; however, clinical trials using EZH2 inhibitors in MRT are still ongoing (ClinicalTrials.gov identifier:).

Recent systems biology-centred studies indicate that loss of other mSWI/SNF subunits, such as AT-rich interactive domain-containing protein 1A (ARID1A), SMARCE1 or the SMARCA4 ATPase, may exhibit mechanistic convergence with SMARCB1 loss[148], particularly in generating and maintaining accessibility over enhancer regions. Coupled with the recent comprehensive architectural characterization of mSWI/SNF complexes[138], these findings provide additional evidence for the mutational patterns observed in human disease and suggest the utility of such studies for other chromatin regulatory complexes. These studies also obviate the need for 3D-structure-based interrogation of disease-associated mutations in these specific components.

Gain-of-function perturbations of mSWI/SNF subunits have also been recently discovered (Fig. 4f). For example, the SS18 (also known as SSXT)–SSX fusion oncoprotein is observed in nearly 100% of cases of synovial sarcoma and integrates as a stable mSWI/ SNF complex subunit[149]. Despite the gain-of-function nature of this event, the SMARCB1 core BAF complex subunit was found to be concurrently displaced from BAF complexes. Recent genome-wide studies defined the targeting profiles of the SS18–SSX-containing complexes, and showed that the SSX tail redirects BAF complexes to new genomic loci and, hence, target genes, at which antagonism of PRC2 facilitates transcriptional activation[150]. These studies support the opposing functions of BAF and PRC2 complexes[151,152] and provide mechanistic evidence for the lack of observed efficacy of EZH2 inhibitors in patients with synovial sarcoma to date[153].

Nevertheless, the promising results from cell line and mouse models have prompted the evaluation of EZH2 inhibitors in clinical trials for mSWI/SNF-perturbed MRT and epithelioid sarcoma, with preliminary positive results[85,154,155]. Inhibitors and ligand-based degrader compounds targeting the bromodomains of the BRD9 and BRD7 mSWI/SNF subunits have been recently developed[156], with BRD9 degradation (and hence ncBAF complex inhibition) showing promise in canonical BAF-perturbed cancers such as synovial sarcoma and malignant rhabdoid tumours[139,157]. The potential for therapeutic efficacy of subunit protein degradation approaches in mSWI/SNF-perturbed cancers has yet to be tested extensively in vivo in disease model systems.

Conclusions and future outlook

Over the past decade, the field of chromatin regulation has made tremendous progress, ranging from understanding the basic chromatin-associated hallmarks of cancer to identifying the underlying genetic changes driving distinct, oncogenic gene expression programmes and promoting tumour development. The challenges and opportunities associated with translating these findings into actionable, mechanism-specific therapeutic strategies have captured the attention of academic research groups and the pharmaceutical industry.

This surge in impactful discoveries continues to be potentiated by the genomic sequencing of human tumours, but also by techniques such as chromatin immunoprecipitation followed by sequencing (ChIP-seq), RNA sequencing (RNA-seq), assay for transposase-accessible chromatin using sequencing (ATAC-seq), Hi-ChIP, Hi-Seq and others, that permit the investigation of chromatin-bound features and topology, modifications, and protein interactions at a genome-wide level. These methodologies are increasingly being paired with advanced computational and bioinformatic approaches. In parallel, state-of-the-art protein identification and mapping approaches based on quantitative mass spectrometry are further elucidating the satellite protein interactome of the chromatin regulatory system described herein. Genetic manipulation strategies, such as CRISPR–Cas9-mediated gene disruption and base editing, as well as major improvements in structural biology approaches, such as cryo-electron microscopy, continue to uncover clinically relevant functional and structural properties of chromatin regulatory proteins and protein complexes. The identification of high-resolution 3D structures of uncharacterized epigenome modifiers and regulators will vastly potentiate both rational design and discovery screens of inhibitors, small molecule or otherwise. Finally, genome-scale RNA interference-based and CRISPR–Cas9-based synthetic lethal studies in hundreds of cancer cell lines and cancer model systems have identified dependencies specific to chromatin regulatory system perturbations, continuing to prompt the development of a wide range of new therapeutic discovery efforts. The systematic, integrative pursuit of such approaches promises a bright future for the further study of the chromatin regulatory system and the elucidation of new therapeutic avenues in cancer.

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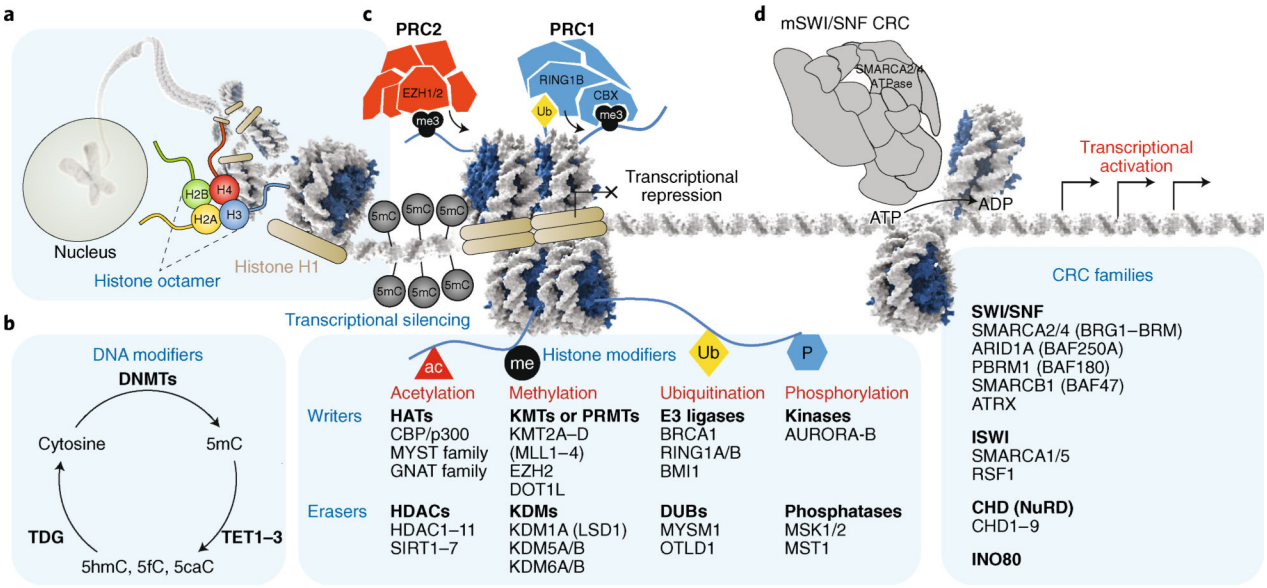

Fig. 1 |. Chromatin regulatory processes in mammalian cells.

a, DNA is wrapped around a histone octamer containing two copies each of histones H2A, H2B, H3 and H4, forming the primary functional unit of chromatin: the nucleosome. Histone H1 binds to DNA at the entry and exit site of the nucleosome. **b**, DNA methylation is achieved by DNMTs, which are responsible for creating the 5mC mark, associated with transcriptional repression, and TET enzymes, which oxidize 5mC to create 5hmC, 5fC and 5caC. **c**, Histone modifications, such as acetylation (ac), methylation (me), ubiquitination (Ub) and phosphorylation (P), serve as instructive marks for both gene activation and gene repression. PRC2 and PRC1 deposit the H3K27me3 and H2AK119Ub marks, respectively, both of which correlate with transcriptional repression. **d**, Four families of ATP-dependent CRCs alter chromatin architecture by mobilizing, depositing or evicting nucleosomes. AURORA-B, Aurora kinase B; BRCA1, breast cancer type 1 susceptibility protein; CBP, CREB-binding protein; DUBs, deubiquitinating enzymes; GNAT, Gcn5-related *N*-acetyltransferases; HATs, histone acetyltransferases; ISWI, imitation SWI; KDMs, methyl demethylases; KMTs, methyl transferases; MSK1/2, mitogen- and stress-activated protein kinase 1/2; MST1, mammalian STE20-like protein kinase 1 (also known as STK4); PBRM1, protein polybromo-1; PRMTs, protein arginine *N*-methyltransferases; RSF1, remodelling and spacing factor 1; SIRT, sirtuin; TDG, thymine DNA glycosylase.

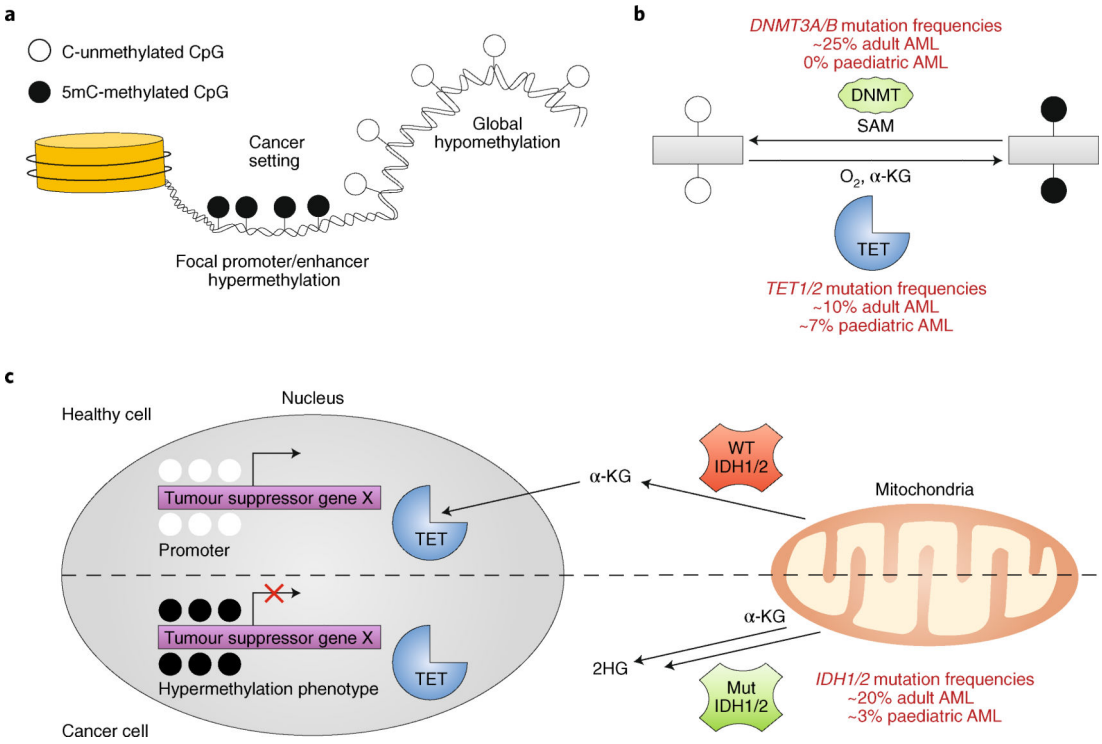

Fig. 2 |. DNMT and TET enzymes and related perturbations in AML.

a, Global hypomethylation and focal promoter/enhancer hypermethylation phenotypes are commonly detected in cancer. **b**, DNMT and TET enzymes are commonly mutated in adult AML and counteract one another via deposition or removal of the 5mC mark, respectively. DNMTs deposit a methyl group on to the carbon-5 position of cytosine using *S*-adenosyl-methionine (SAM) as a substrate, and TET enzymes rely on α-ketoglutarate (α-KG) and oxygen to oxidize 5mC and promote cytosine demethylation. **c**, *IDH1/2* (encoding isocitrate dehydrogenase 1) mutations, which are common in AML, inhibit TET activity by converting the TET substrate α-KG to 2-hydroxyglutarate (2HG), resulting in a hypermethylation phenotype. WT, wild type.

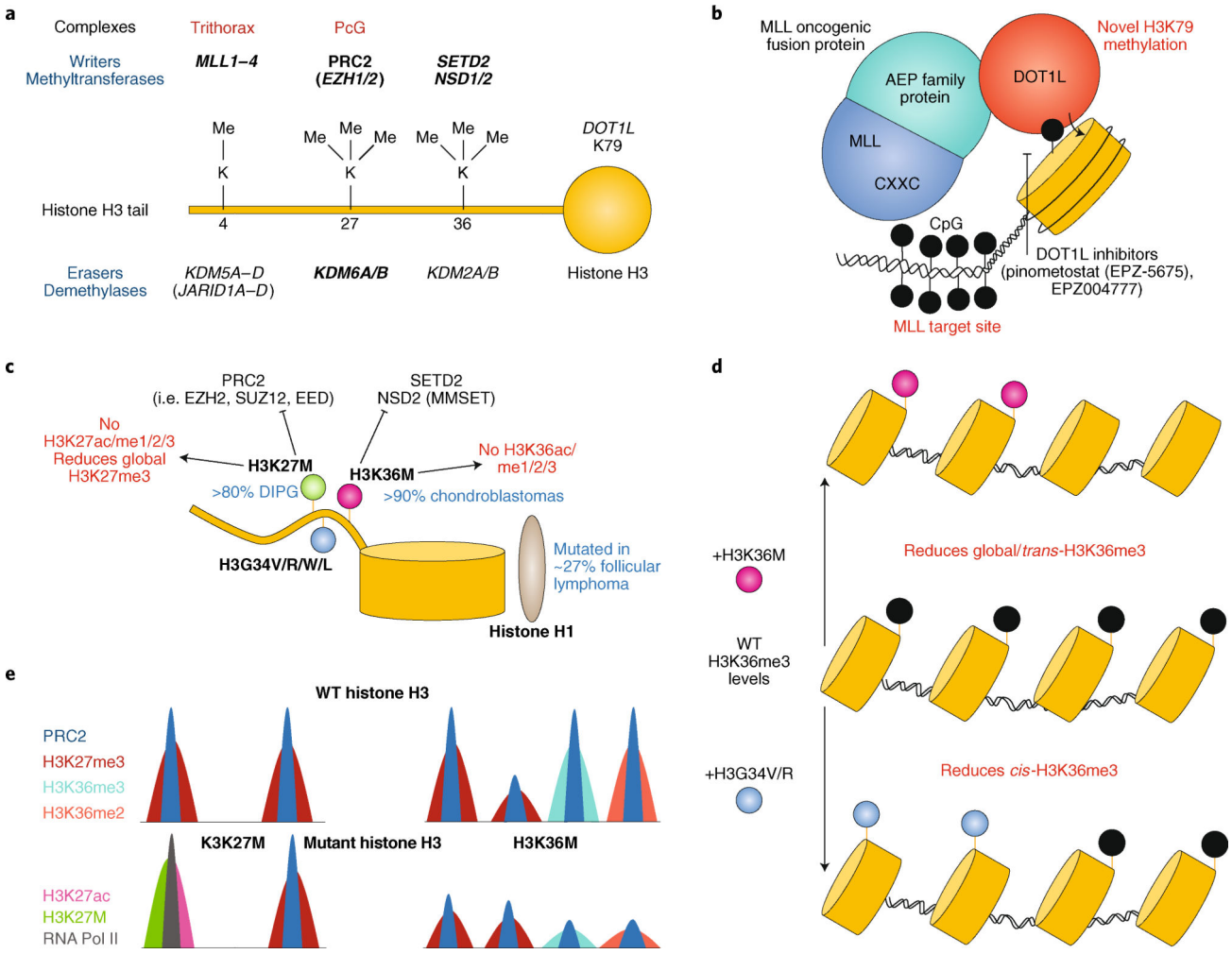

Fig. 3 |. Histone H3 methylation modifications and disruption in cancer.

a, Histone H3 methyltransferases and demethylases. Mutations to genes in bold are implicated in cancer. **b**, Depiction of MLL–ENL-rearranged leukaemia. The MLL CXXC domain targets the fusion protein to MLL target sites and the ENL domain recruits DOT1L methyltransferase activity, resulting in aberrant methylation. **c**, Schematic of oncohistone mutations in cancer and their antagonism with methyltransferases. **d**, The H3K36M mutation results in the global reduction of H3K36me3 levels, whereas the H3K34 mutation diminishes only *cis*-H3K36me3 levels. **e**, Schematic of H3K27M and H3K36M oncohistone chromatin occupancy compared to wild-type (WT) histone H3. H3K27M inhibits H3K27me3, resulting in RNA polymerase II (RNA Pol II) recruitment and activation, as assessed by H3K27ac levels. The H3K36M mutation reduces genome-wide H3K36me2/3 and H3K27me3 levels. DIPG, diffuse intrapontine glioma.

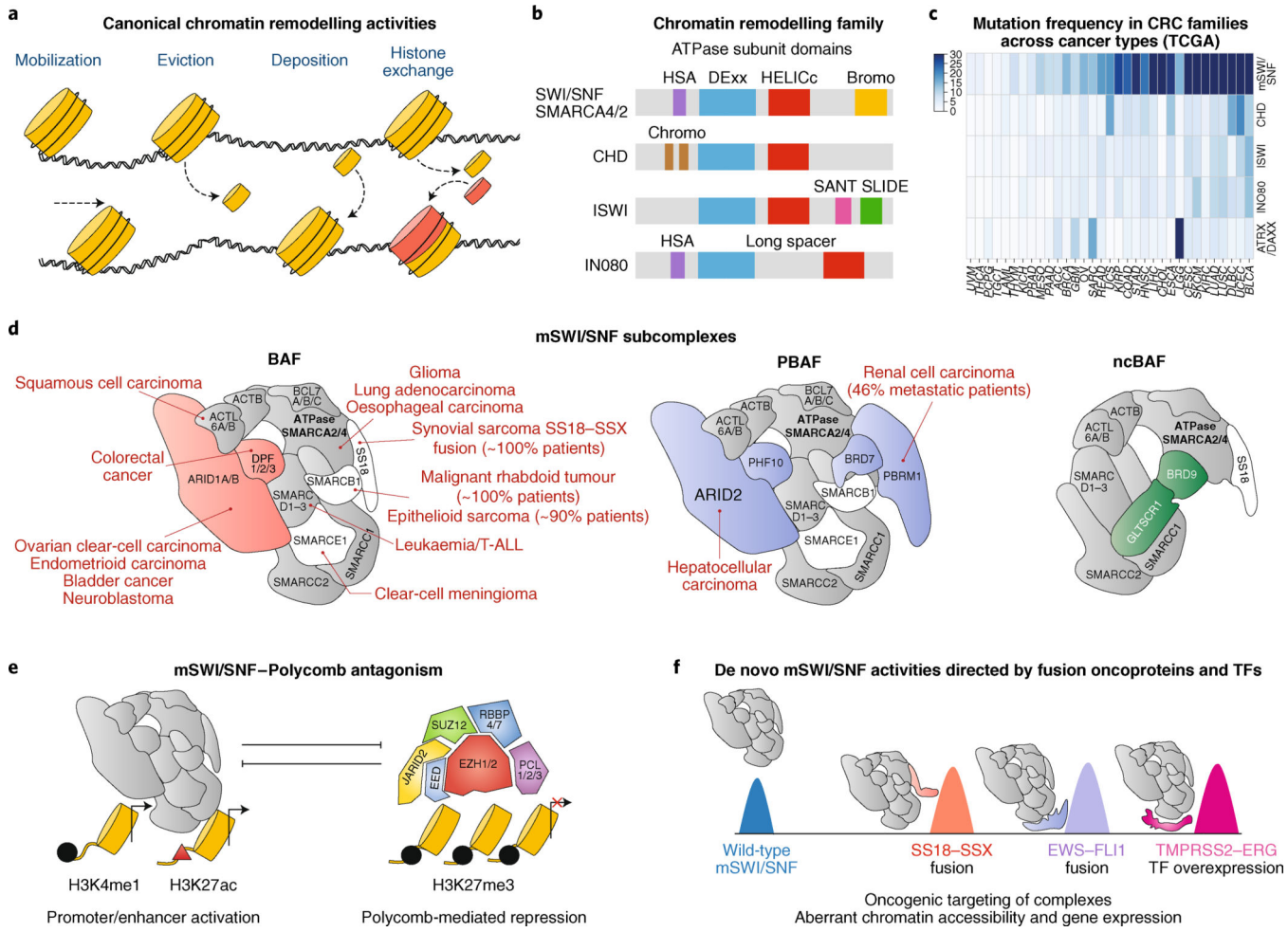

Fig. 4 |. CRCs in cancer: a focus on mSWI/SNF (BAF) complexes.

a, Cartoon depiction of the chromatin remodelling activities: nucleosome sliding, ejection and placement, and histone variant exchange. **b**, Domain organization within the ATPase subunit of each class of CRCs. **c**, Pan-cancer mutation frequency across chromatin remodelling families. Mutation frequencies for all genes encoding members of each family were summed and represented as a heatmap. Analysis of public The Cancer Genome Atlas (TCGA) data for 33 available cancer types showing mutation frequency rates across 4 CRC families and SWI/SNF-like ATRX/DAXX. **d**, mSWI/SNF subcomplex protein associations overlayed with subunit-specific mutations identified across cancer types. The mSWI/SNF subcomplex-defining subunits are coloured in red (BAF), purple (PBAF) and green (ncBAF). **e**, mSWI/SNF complexes are typically associated with active chromatin landscapes and directly oppose Polycomb-mediated repression. **f**, Gain-of-function perturbations to mSWI/SNF complexes include fusion oncoproteins and transcription factors (TFs) that tether to mSWI/SNF complex surfaces. The SS18–SSX fusion oncoprotein replaces the SS18 subunit to hijack complexes genome wide, the EWS–FLI1 (friend leukaemia integration 1 transcription factor) fusion directs complexes to GGAA repeat sites in Ewing sarcoma and the ERG transcription factor targets BAF complexes to ETS DNA sequence motifs genome wide, each of which results in aberrant, cancer-specific

transcriptional regulation. ACTB, actin, cytoplasmic 1; ACTL6, actin-like protein 6; BCL7, B-cell CLL/lymphoma 7 protein family member; CHD, chromodomain helicase DNA-binding; DPF, zinc-finger protein neuro-d4; GLTSCR1, BRD4-interacting CRC-associated protein; HELICc, helicase superfamily C-terminal; HSA, helicase/SANT associated; PCL, Polycomb-like protein; PHF10, PHD finger protein 10; SLIDE, SANT-like but with several insertions; T-ALL, T cell acute lymphoblastic leukaemia; TMPRSS2, transmembrane protease serine 2.