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Epigenetic modifiers: activities in renal cell carcinoma

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Abstract

Renal cell carcinomas (RCCs) are a diverse set of malignancies that have recently been shown to harbour mutations in a number of chromatin modifier genes — including *PBRM1*, *SETD2*, *BAP1*, *KDM5C*, *KDM6A,* and *MLL2* — through high-throughput sequencing efforts. Current research focuses on understanding the biological activities that chromatin modifiers employ to suppress tumorigenesis and developing clinical approaches that take advantage of this knowledge. Unsurprisingly, several common themes unify the functions of these epigenetic modifiers, particularly regulation of histone post-translational modifications and nucleosome organization. Furthermore, chromatin modifiers also govern processes crucial for DNA repair and maintenance of genomic integrity, as well as the regulation of splicing and other key processes. Many chromatin modifiers have additional noncanonical roles in cytoskeletal regulation, which further contribute to genomic stability, expanding the repertoire of functions that might be essential in tumorigenesis. Our understanding of how mutations in chromatin modifiers contribute to tumorigenesis in RCC is improving, but remains an area of intense investigation. Importantly, elucidating the activities of chromatin modifiers offers intriguing opportunities for the development of new therapeutic interventions in RCC.

Introduction

Renal cell carcinoma (RCC) is among the top ten most commonly diagnosed malignancies in the United States, with an estimated 63,990 new cases having been diagnosed in 2018[1]. RCC is a heterogeneous disease that includes several histological subtypes; the most common histology is clear cell renal cell carcinoma (ccRCC), which represents up to 75% of RCCs, whereas papillary RCC (pRCC), chromophobe renal cell carcinoma (chRCC) and several rare tumour types such as renal medullary carcinoma comprise the remaining ~25% (REF. [2]). Major therapeutic advances in the treatment of metastatic RCC have been made over the past two decades, mainly owing to the advent of antiangiogenic targeted therapies and immunotherapies. However, only a fraction of patients show durable clinical responses and long-term remission[3].In addition, the long-term prognosis for all patients with relapsed

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and metastatic disease remains poor[4]. Thus, an urgent clinical need exists for novel insights and therapeutic strategies.

The necessity for novel therapeutic strategies in RCC has motivated considerable research dedicated to dissecting the molecular biology underpinning this unique group of cancers. The discovery of mutations in the gene encoding von Hippel-Lindau disease tumour suppressor (*VHL*) in ccRCC was a major step forward[5]. Somatic and germline inactivating *VHL* mutations, as well as deletion of *VHL*, are present in a very large proportion of ccRCCs[6–11]. Loss of VHL function results in the inappropriate stabilization of hypoxia inducible factor (HIF), which triggers a pseudohypoxic response that includes a profound upregulation of angiogenesis[12]. This understanding inspired the clinical application of angiogenesis inhibitors in ccRCC, which now represent a clinical standard of care[13]. However, the field has struggled with the identification of co-drivers, given that *VHL* mutations are necessary, but not sufficient, for ccRCC tumorigenesis[14,15].

In 2010, the targeted sequencing of 3,544 protein coding genes in 101 ccRCC samples uncovered mutations in four genes encoding histone modifying enzymes, namely: histone-lysine N-methyltransferase SETD2 (*SETD2*), a histone H3 lysine 36 methyltransferase; lysine-specific demethylase 5C (*KDM5C*; also known as *JARID1C*), a histone H3 lysine 4 demethylase; histone-lysine N-methyltransferase 2D (*KMT2D*; also known as *MLL2*), a histone H3 lysine 4 methyltransferase; and lysine-specific demethylase 6A (*KDM6A*; also known as *UTX*), a histone H3 lysine 27 demethylase[16]. The following year, mutations in protein polybromo-1 (*PBRM1*; also known as *BAF180*), ubiquitin carboxyl-terminal hydrolase BAP1 (*BAP1*), AT-rich interactive domain-containing protein 1A (*ARID1A*; also known as *BAF250A*) and *ARID1B* (also known as *BAF250B*) genes were identified in ccRCC[17,18]. BAP1 is a deubiquitinase that targets mono-ubiquitylation of lysine 119 on histone H2A (H2AK119ub1), whereas PBRM1, ARID1A and ARID1B are components of the switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling complex, which is involved in nucleosome repositioning[19]. Several large-scale sequencing studies have since confirmed these findings and have further characterized the repertoire of genetic alterations, not only in large numbers of ccRCCs, but also in pRCCs and chRCCs[6,7,9,20,21]. These enzymes all share a common theme — they participate in modifying chromatin structure.

In this Review, we focus on requisite co-drivers in RCC, which share common chromatin modifying activity and discuss how mutations in their encoding genes contribute to the development and progression of this disease. We cover key aspects of chromatin modifier biology and the repertoire of mutations in these genes in RCC, and discuss the canonical and noncanonical functions of chromatin modifiers and how their dysfunction contributes to this disease. Finally, we examine potential therapeutic opportunities created by loss-of-function of chromatin modifiers.

Chromatin modifiers in RCC

When eukaryotic cells are not dividing, their DNA is assembled around core histones (H2A, H2B, H3 and H4 family proteins), the basic structural units of the nucleosome, and packaged along with other protein complexes within the nucleus; the resulting complex macromolecule is called chromatin[22]. Linker histones attach to core histones at DNA

entry and exit sites and function to mediate the arrangement of nucleosomes with respect to both each other and to their associated gene units. Access to nucleosomal DNA is further mediated by the action of chromatin remodeling factors, which are in turn aided by a complex coding system of post-translational modifications (PTMs) present on the histones[23]. These PTMs — including methylation, acetylation, phosphorylation, ubiquitylation, sumoylation, citrullination and ADP-ribosylation — occur in the long C-terminal tail domains of histones (which protrude from the nucleosome), altering their interaction with DNA and nuclear proteins. Histone PTMs and their associated functions are dynamically regulated by numerous enzymes that can be classified as 'writers' (enzymes that add PTMs), 'erasers' (enzymes that remove PTMs) and 'readers' (interacting proteins that recognize the histone PTM). This combination of nucleosome position and associated PTMs form a 'histone code' that can be interpreted by other proteins to elicit a variety of biological functions[23]. Thus, chromatin organization provides an additional layer of transcriptional regulation by controlling DNA accessibility. This remarkable architecture enables the dynamic genomic regulation that is necessary for a cell to orchestrate its regular functions.

Chromatin remodelers—When wrapped around histones, DNA is less accessible for transcription, as well as other processes; thus, DNA accessibility must be controlled by nucleosomal remodeling complexes, which interact with PTMs to guide localization and subsequently restructure chromatin by moving, destabilizing and ejecting nucleosomes[24]. To date, four families of chromatin remodelers have been identified — SWI/SNF, imitation switch (ISWI), chromodomain helicase DNA-binding (CHD) and INO80[25].

Alterations in components of the SWI/SNF chromatin remodeling complex are frequently observed in RCC[7,18,20,21], as well as in other cancers[26–30]. SWI/SNF complexes are assembled around a catalytic subunit — protein brahma homolog (BRM; encoded by *SMARCA2*) or transcription activator BRG1 (encoded by *SMARCA4*) — that provides energy for chromatin remodeling through ATP hydrolysis[31] (FIG. 1). The BRM subunit is incorporated into a complex referred to as the BRM-associated factor (BAF) complex, whereas the BRG1 subunit can nucleate either BAF or polybromo-associate BAF (PBAF) complexes. The BAF and PBAF complexes differ in the composition of several subunits, including those involved in chromatin targeting, which are thought to mediate recruitment of the complex to specific chromatin regions. The ARID1A and ARID1B subunits are believed to participate in the targeting of BAF complexes[32,33], whereas the AT-rich interactive domain-containing protein 2 (ARID2; also known as BAF200)[34], bromodomain-containing protein 7 (BRD7)[35] and PBRM1[36] subunits are thought to mediate PBAF complex targeting to DNA.

PBRM1.: The PBRM1 subunit contains six tandem bromodomains, two bromo-adjacent homology (BAH) domains and a high-mobility group (HMG) domain (FIG. 2A). Bromodomains, composed of ~100 amino acids, recognize acetylated lysine residues on histone tails, a mark associated with active transcription[37]. Each bromodomain of PBRM1 has a distinct pattern of affinity for specific acetylated peptides on histones[37]. Although the overall affinity of each bromodomain for a peptide is low, PBRM1 is thought to bind

cooperatively to a precise pattern of acetylated lysine residues in nucleosomes[38]. Thus, this process in part mediates PBAF complex recruitment to specific regions of DNA where nucleosome remodeling occurs.

The importance of BAH domains and bromodomains for tumour suppressive function is emphasized by the high frequency of *PBRM1* missense mutations in ccRCC[18,39] (FIG. 2A). Mutations in other SWI/SNF complex subunits have also been described in RCC, such as *ARID1A*[6,7,20,21,40–42]. Notably, *ARID1A and ARID1B* mutations have been observed in conjunction with *PBRM1* mutations[18], suggesting that dysfunction of these genes cooperate in tumorigenesis. The potential implications of BAF and PBAF complexes in RCC is also exemplified by the presence of mutations in the catalytic BRG1 subunit, which forms part of both complexes[43].

The mechanisms by which mutations in PBRM1 or ARID1A and ARID1B contribute to tumorigenesis in RCC is incompletely understood. PBRM1-mutated RCCs have been found to have a characteristic gene expression signature[44], suggesting that PBRM1 functions as a tumour suppressor as part of the targeting subunit of the PBAF nucleosome remodeling complex through its effects on DNA accessibility and gene expression. In this manner, PBRM1 and the SWI/SNF complex have been implicated in the regulation of diverse biological pathways, from hypoxia to interferon signaling[45–49]. For instance, loss-of-function of PBRM1 has been associated with favourable clinical outcomes with immune checkpoint therapy in RCC[48] and melanoma[50]. How these pathways contribute to tumourigenesis and progression are just now beginning to be recognized.

BAP1—BAP1, a nuclear localized deubiquitinase enzyme and a tumour suppressor protein, is also frequently mutated in ccRCC[39]. BAP1 belongs to the ubiquitin C-terminal hydroxylase (UCH) family of deubiquitinases, which mediate the binding and cleavage of the ubiquitin isopeptide bond[51]. The PTM of proteins by covalent attachment of ubiquitin controls many essential cellular processes, such as targeting proteins for assembly into complexes, transport and degradation[52]. BAP1 is comprised of several functional domains, including the ubiquitin C-terminal hydrolase (UCH) region, the BARD1 binding domain, the HCF-C1 binding motif (HBM), the HBM-like motif, the BRCA1-binding domain and two putative nuclear localization signals (NLS1 and NLS2) (FIG. 2B). In addition, BAP1 contains a region of extreme acidity, multiple potential phosphorylation sites and N-linked glycosylation sites. BAP1 has been shown to interact with the INO80 family of chromatin remodelers, but its role in mediating nucleosome remodeling remains to be fully developed [53].

Classically, BAP1 is known to deubiquitinate H2AK119ub1, which is associated with polycomb-mediated gene repression[54] (FIG. 1). Polycomb proteins form part of two complexes — the polycomb repressive complex 1 (PRC1) and PRC2. The PRC2 complex can trimethylate lysine 27 on histone H3 (H3K27me3) via the histone-lysine N-methyltransferases EZH1 or EZH2[55]. The PRC1 complex mediates mono-ubiquitylation of histone H2A at lysine 119 (H2AK119ub1)[56]. The traditional view on polycomb-mediated gene silencing involves a consecutive model whereby PRC2 first trimethylates H3K27, followed by the binding of PRC1 to H3K27me3 and the subsequent addition of

the H2AK119ub1 mark[55,57]. Noncanonical PRC1 complexes have been found to target chromatin independently of H3K27me3[58,59]. For instance, noncanonical PRC1 targeting to chromatin has been shown to occur through the activity of lysine-specific demethylase 2B (KDM2B), which specifically targets nonmethylated CpG islands via its CxxC domain[60]. In addition, noncanonical PRC1 complexes can also induce H2AK119ub1 to initiate PRC2 recruitment and the subsequent placement of H3K27me3[61].

SETD2—Mutations in the histone H3 lysine 36 (H3K36) trimethyltransferase SETD2 have been reported in ccRCC[16] (FIG. 2C). Several histone methyltransferases have been shown to mediate H3K36 monomethylation and dimethylation, whereas SETD2 is the only enzyme responsible for H3K36 trimethylation (H3K36me3) in mammalian cells[62]. The complexity of H3K36 regulation in higher eukaryotes suggests that this PTM has an important regulatory function.

The human SETD2 protein consists of several functional domains: the methyltransferase (SET), the SET2-Rpb1 interacting (SRI) and WW domains (FIG. 2C). The catalytic activity of the SET domain mediates histone methyltransferase activity[63]. The SRI domain binds to diphosphorylated serine 2 and serine 5 residues on the carboxyl-terminal domain (CTD) repeats of RNA polymerase II (RNA Pol II) [64], a domain with an important role in transcriptional regulation[65]. Unphosphorylated RNA Pol II is associated with assembly of the preinitiation complex; however, phosphorylation of RNA Pol II at serine 2 and serine 5 elicits promoter clearance and elongation, allowing other factors necessary for transcriptional elongation and termination to bind the CTD[65]. SETD2 preferentially binds to phosphorylated RBP1; thus, it travels along with RNA Pol II and is available to trimethylate H3K36 in actively transcribed genes.

KDM5C and KDM6A—Mutations in other chromatin modifier genes, such as *KDM5C* and *KDM6A*, have been identified in ccRCC[16,18]. Located on chromosome Xp11.22 and Xp11.3, *KDM5C* and *KDM6A* respectively code for demethylases that target trimethylated lysine 4 on histone H3 (H3K4me3) and H3K27me3. KDM5C has been described as a tumour suppressor in ccRCC. Overexpression of KDM5C, which is a HIF target gene, decreased the global H3K4me3 levels, suppressed global gene transcription and retarded in vivo tumour growth in xenograft models[66]. KDM5C also contributes to the maintenance of heterochromatin and directly binds to satellite repeats during heterochromatin duplication in S phase[67]. In the latter study, tumours harbouring *KDM5C* mutations from patients with ccRCC showed features of heterochromatin disruption and genomic rearrangement, and these patients had a poor prognosis[67].

Repertoire of mutations in ccRCC—In ccRCC, mutations in *PBRM1 or SETD2* and *BAP1* have been described to be mutually exclusive, whereas mutations in *PBRM1* and *SETD2* have the tendency to co-occur[11,39,68]. Patients with RCC whose tumours harbour a *BAP1* mutation have pathological features associated with aggressive disease, such as rhabdoid histology and tumour infiltration, and consistently have poorer survival outcomes than those with mutations in *PBRM1*[39,44,69,70]. By contrast, evidence regarding the clinical significance of mutations in *PBRM1* and *SETD2* in RCC is inconsistent[39,44,69,70]. A study from 2017 reported that loss or low expression of SETD2, but not H3K36me3, by

immunohistochemistry, was associated with a poor prognosis in patients with ccRCC[71]. Initially, PBRM1 mutation was reported to be associated with increased aggressiveness in ccRCC[69]. In addition, loss of PBRM1 expression by immunohistochemistry was associated with advanced stage, high Furman grade and poor overall survival[72]. However, other reports provide evidence suggesting that ccRCCs have similar rates of *PBRM1* mutations regardless of stage and do not seem to influence patient survival outcomes[44,70,73]. The high prevalence of this mutation, and its inclusion as a truncal event, suggests that later events in tumour progression have a greater degree of influence on the ultimate behavior of the tumour.

Recently, the Tracking Renal Cell Cancer Evolution Through Therapy (TRACERx Renal) study examined the association between disease stage and clinical outcomes in a large cohort of primary ccRCC tumours[11]. Using multi-region genomic profiling to study tumour heterogeneity in ccRCC, the authors identified seven major evolutionary subtypes in ccRCC, namely: multiple clonal drivers; VHL wild-type; VHL monodriver; BAP1-driven (VHL→BAP1); VHL→PBRM1→SETD2; VHL→PBRM1→phosphoinositide 3-kinase (PI3K); and VHL→PBRM1→somatic copy number alteration (SCNA)[11]. Consistent with other reports[74], the multiple clonal driver subtype, characterized by the clonal co-occurrence of drivers that are usually mutually exclusive (such as BAP1 and PBRM1, or BAP1 and SETD2), was associated with aggressive disease and comprised the largest tumours in the cohort[11]. The majority of tumours in the BAP1-driver (VHL→BAP1) mutational subtype had no other detectable mutational drivers, which confirmed the tendency of mutual exclusivity between BAP1 and PBRM1 mutations[68], and suggested that BAP1 mutations drive strong clonal expansion[11]. Conversely, tumours in the the VHL→PBRM1→SETD2 subtype had extensive clonal branching and a preponderance for parallel evolution[11]. Although mutations in PBRM1 seem to be an early event, with evidence showing clonality of up to 74%, SETD2 mutations seem to occur later during RCC development, given that subclones with mutations in SETD2 showed limited expansion[11]. Interestingly, the spatial clustering of parallel SETD2 mutations suggested a potential role for SETD2 loss in niche-specific clone selection[11]. Importantly, these evidences suggest that the order in which driver events are acquired can have prognostic and therapeutic implications. These findings could in part explain the discrepancies regarding the clinical significance of mutations in SETD2 and PBRM1 in previous studies, which involved analysis of single tumour samples. This study[11] and others[75–78] indicate that multi-region sampling of RCCs might be necessary to account for tumour heterogeneity.

Co-drivers in RCC mouse models—In mice, loss of *Vhl* alone does not induce kidney cancer and additional mutations in co-drivers were, therefore, believed to be necessary for tumorigenesis[79–81]. Initial efforts to explore the potential roles of *Pbrm1*, *Bap1* and *Setd2* as co-drivers in mouse models were hampered because homozygous loss of these genes resulted in embryonic lethality[36,82–84]. It was not until *Vhl* and *Bap1* were conditionally knocked out that mice developed kidney tumours[85,86]. Conditional knockout of *Vhl* and *Pbrm1* was also shown to cause ccRCC, as well as large cysts, in mouse kidneys[47,85]. However, the conditional knockout of *Setd2* and *Vhl* has not been reported to date. In mice, concurrent knockout of *Vhl* and *Bap1* produced high-grade ccRCC tumours, whereas concurrent *Vhl* and *Pbrm1* knockout was associated with the development of low-grade

ccRCC[85]. These findings provide further support to the co-driver hypothesis, which posits that a *VHL* loss-of-function mutation alone is insufficient to promote tumorigenesis, but that a second mutation is required for the acquisition of malignant potential.

Mutations in other RCC histologies—Mutations in chromatin modifiers are not unique to ccRCC and have been detected in other RCC histologies. Although rare, type 1 pRCC tumours have been shown to harbour inactivating mutations in *KDM6A* and *SMARCB1* (also known as BAF47, INI1 or *SNF5*; encoding SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1)[7]. Conversely, type 2 pRCC is associated with loss of *PBRM1*, *BAP1*, *SETD2* and *ARID2*, although the frequencies are lower than those observed in ccRCC[87,88]. Interestingly, as observed in ccRCC, mutations in *PBRM1* and *BAP1* are mutually exclusive whereas mutations in *PBRM1* and *SETD2* tend to co-occur in pRCC[7,68]. Although loss of *PBRM1*, *BAP1* and *SETD2* is common to both ccRCC and type 2 pRCC, the path to tumorigenesis seems to proceed via different routes. Notably, loss of chromosome 3p (a consistently large deletion which encompasses *VHL*, *PBRM1*, *BAP1* and *SETD2*) is rarely observed in pRCCs and inactivation of *PBRM1*, *BAP1* and *SETD2* occurs primarily through mutation[10,11,76,77,87]. Thus, pRCCs are often heterozygous for these genes, suggesting that *PBRM1*, *BAP1* and *SETD2* genes are haploinsufficient, such that a loss-of-function mutation in one gene copy cannot be fully compensated by the presence of the remaining wild-type allele. In chRCC and renal oncocytomas, mutations in *PBRM1*, *BAP1* and *SETD2* are rare[88].

The involvement of these chromatin modifiers has also been described in sarcomatoid RCCs. Mutations in *PBRM1*, *BAP1* and *SETD2* were detected in sarcomatoid ccRCC, tumours characterized by an unusually high prevalence of *TP53* mutations compared with conventional ccRCCs[89,90]. Similar to their ccRCC counterparts, sarcomatoid ccRCCs also harbour mutations in *VHL*, consistent with the observation. However, unlike conventional ccRCCs, sarcomatoid ccRCCs rarely have loss of chromosome 3 and are less likely to harbour *PBRM1* mutations[90]. Furthermore, these findings also indicate that IHC might not be an appropriate method to screen for mutations in these proteins (which result in altered expression) in non-ccRCC tumours, and that genomic sequencing would be more suitable.

Canonical functions in RCC

Chromatin and chromatin modifiers mediate many important processes ranging from transcription and splicing to DNA repair. Furthermore, histone PTMs can regulate other epigenetic marks, for instance, via crosstalk with DNA methylation. Dysregulation of the canonical functions (DNA damage repair, transcriptional regulation, splicing and epigenetic crosstalk) of PBRM1, BAP1 and SETD2 contribute to the development and progression of RCC.

DNA damage repair—Cancer cells, including RCC, are known to have defects in the DNA mismatch repair (MMR) and DNA double-strand break repair (DSB repair) pathways[91,92]. Accordingly, defective MMR function and impaired DSB repair enables elevated mutation frequencies and can lead to certain cancers[93]. As DNA repair must occur

in the context of chromatin, the processes of DNA repair and chromatin modification are intimately linked.

SETD2 and H3K36me3 in DNA repair.: Several studies have implicated SETD2 and its cognate PTM, H3K36me3, in both DNA MMR and the resolution of DSBs. In human cells, DNA mismatches are recognized by the MutSα (MSH2–MSH6 heterodimer) and MutSβ (MSH2–MSH3 heterodimer) complexes, triggering additional MMR components to repair the DNA mismatch[94,95]. Given that MMR (and most forms of DNA repair) is preferentially directed to actively transcribed genes, the H3K36me3 mark, which is associated with actively transcribed genes, might enable this preferential activity. Accordingly, MutS protein homolog 6 (MSH6) contains a PWWP domain that has been shown to bind to H3K36me3 and promote the recruitment of MutSα complexes to genomic regions that contain coding sequences[94,96] (FIG. 3A). These discoveries have shed light on how the histone code contributes to the high fidelity of DNA replication in eukaryotic cells by enhancing MMR efficiency at coding regions, where it is the most crucial.

DNA DSBs are severe events in eukaryotic cells that can arise from genotoxic stress and that can be repaired through homologous recombination (HR) or non-homologous end joining (NHEJ)[92]. H3K36me3 can mediate DSB repair through its interaction with PHD finger protein 1 (PHF1) (FIG. 3B). The Tudor domain of PHF1 binds to H3K36me3 and stabilizes PHF1 at DNA DSBs, together with the X-ray repair cross-complementing protein 6 (Ku70)–X-ray repair cross-complementing protein 5 (Ku80) heterodimer and poly ADP-ribose polymerase 1 (PARP1), which constitute the DNA strand break recognition complex[97]. In addition to focusing this activity to actively transcribed genes, the binding of PHF1 to H3K36me3 promotes an open chromatin state (euchromatin), which is also necessary for the successful resolution of DSBs.

The activity of two other H3K36me3 readers, lens epithelium-derived growth factor (LEDGF; also known as PSIP1) and mortality factor 4-like protein 1 (MORF4L1; also known as MRG15) were also shown to promote DSB repair (FIG. 3B). Following DNA damage, MORF4L1 interacts with partner and localizer of BRCA2 (PALB2) to activate the HR pathway[98]. LEDGF stimulates CtIP-mediated DNA end resection, a critical step during HR-mediated repair of DNA DSBs[99,100], and also promotes DSB repair through the recruitment of histone acetyltransferase KAT5 to chromatin after DNA damage, which catalyzes the acetylation of lysine 16 on histone H4 (H4K16ac)[101]. This acetylation suppresses chromatin condensation and, therefore, promotes a favourable euchromatic environment for the resolution of DSBs[102]. The crosstalk between H3K36me3 and H4K16ac truly emphasizes the complexity and elegance of the histone code.

Interestingly, the differential modification of H3K36, including either methylation by Set2 (the yeast SETD2 homolog) or acetylation by histone acetyltransferase Gcn5 (the yeast KAT2A homolog), has been suggested to coordinate the choice of DSB repair pathway (that is, HR or NHEJ) in yeast[103]. In both yeast and mammals, modification of H3K36 is regulated during the cell cycle; H3K36me3 levels peak in G1 phase, during which NHEJ predominates, whereas H3K36 acetylation peaks in late S/G1 phase, during which HR occurs[94,103]. Cancer cells are known to compensate for defects in one strategy of DNA

repair by using alterative repair pathways[104]. The differential preference for DNA repair pathways mediated by modification of H3K36 could in part explain why a drug synthetic lethality screen did not show the increased sensitivity of SETD2 mutant RCC cells to PARP inhibitors, DNA damaging drugs (such as etoposide) or microtubule toxins (such as taxanes and vinca alkaloids), compared with RCC cells harbouring wild-type SETD2[105].

SETD2 loss and DNA damage repair in RCC.: Unsurprisingly, SETD2 loss has been reported to compromise DNA damage repair in RCC cell lines and in tumour samples. Following exposure to ionizing radiation, SETD2-depleted RCC cells showed delayed resolution of phosphorylated histone H2AX (γH2AX) foci, which marks ongoing DNA damage signalling and DSBs[100,106]. Furthermore, levels of H3K36me3 and SETD2 inversely correlated with γH2AX staining in human RCC tumour samples[107]. In addition, SETD2-depleted RCC cells showed reduced formation of DNA repair protein RAD51 homolog 1 (RAD51) foci following induction of DNA damage with ionizing radiation[100,107]. Chromatin immunoprecipitation sequencing (ChIP-seq) revealed that SETD2-deficient tumours from patients with RCC had an increased co-occurrence of chromosomal breaks and point mutations at sites marked by H3K36me3 compared with normal tissues[107]. These findings suggest that under normal physiological conditions, sites marked with H3K36me3 are less prone to having unrepaired DNA damage than other genomic locations that lack this modification, and that SETD2 loss contributes to disproportionate DNA damage in transcribed genes, which might be more relevant to the development of cancer.

Transcriptional regulation—Abnormal splicing patterns, although not well understood, are frequently associated with human disease, and are a hallmark of cancer[108]. Alternative splicing can occur through five modes, namely, exon skipping, mutually exclusive exons, alternative donor site, alternative acceptor site and intron retention, all of which predominantly occur co-transcriptionally. Chromatin modifiers and their associated histone PTMs are known to recruit other nuclear factors that mediate not only splicing, but also transcriptional elongation and the transcriptional response to stimuli.

H3K36me3 patterning in splicing.: The pattern of H3K36me3 distribution is recognized as an important factor in the governance of exon utilization. Indeed, lower levels of the H3K36me3 modification have been reported in alternative exons compared with constitutive exons[109]. Moreover, the presence of splicing factor 3B subunit 3 (SF3B3), a splicing factor that is enriched for at exon–intron junctions, was reported to influence the distribution of H3K36me3 marks[109,110]. On average, alternative exons assemble fewer spliceosomes than constitutive exons, which suggests that the activity of the spliceosome is influenced by the H3K36me3 modification[111].

In addition to its roles in preventing cryptic transcription and promoting elongation, the H3K36me3 reader MORF4L1 has also been shown to influence splicing (FIG. 4A). MORF4L1 has been shown to cooperate with polypyrimidine tract-binding protein 1 (PTBP1) to promote exon inclusion[112]. In addition, MORF4L1 has also been reported to recruit lysine-specific demethylase 5B (KDM5B) — which targets H3K4me3, a modification associated with promoter regions of transcribed genes — to H3K36me3-

marked intergenic regions to promote the removal of H3K4me3[113]. Thus, the interaction between H3K36me3, MORF4L1 and KDM5B ensures transcriptional fidelity by repressing cryptic intergenic transcription, whereas the MORF4L1–PTBP1 interaction mediates splicing through the regulation of alternative exon inclusion (FIG. 4A).

Another H3K36me3 reader, zinc finger MYND domain-containing protein 11 (ZMYND11; also known as BS69), has been reported to regulate intron retention (FIG. 4B)[114]. Often, intron-retaining transcripts contain a premature stop codon, which triggers the nonsense-mediated decay pathway (NMD pathway), and therefore, is associated with decreased gene expression. In this context, ZMYND11 functions as a tumour suppressor by promoting intron retention in certain target genes, which are generally those with high expression and potential oncogenic functions.

Experimental evidence indicates that in the setting of SETD2 deficiency, these processes are in fact disrupted in ccRCC. Extensive genomic analysis has demonstrated that human ccRCC tumours harboring *SETD2* mutations had more open chromatin, as measured by formaldehyde-assisted isolation of regulatory elements sequencing (FAIRE-seq) compared to either SETD2 wild-type tumours or matched normal tissue, and that regional chromatin opening was highly correlated with gene expression[15]. In addition, these same regions also displayed a higher frequency of alternate exon utilization as well as intron retention, suggesting an error-prone splicing process.

PBRM1 loss in hypoxia signalling.: Reports of the effects of PBRM1 loss on hypoxia signalling are inconsistent. In VHL-deficient RCC cells, PBRM1 loss has been described to both enhance and suppress hypoxia signalling[46,115]. The differential effect of PBRM1 loss on response to hypoxia seems to be cell-type specific and depends on the expression of expression VHL, HIF-1α and HIF-2α in RCC cells. In RCC, HIF-1α has long been thought to function as a tumour suppressor and is often deleted, whereas HIF-2α has been considered the primary oncogenic driver. Importantly, there is a consensus that PBRM1 functions as a tumour suppressor by facilitating the expression of HIF-1α target genes[45,46,115]. This hypothesis is in agreement with the observation that the SWI/SNF complex is required for expression of HIF-1α target genes and HIF-1α-induced cell cycle arrest in other cell types[116]. However, owing to discrepancies in these studies, whether PBRM1 is also required for expression of HIF-2α target genes is unclear. Nonetheless, it seems that PBRM1 loss contributes to ccRCC development and progression by downregulation of HIF-1α signalling.

Epigenetic crosstalk—Epigenetic modifications do not function alone, but rather work together in various combinations and can regulate one another, which diversifies their function. The result is a complex, but elegant, regulatory network. However, aberrations in chromatin modifiers, as well as loss or aberrant patterning of histone PTMs, frequently has far-reaching consequences that can further derange other epigenetic states.

Histone methylation.: As mentioned above, actively transcribed genes are marked by H3K36me3 in gene bodies and by H3K4me3 at promoters near the transcription start site. Repressed genes are characterized by H3K27me3, which is catalyzed by the PRC2 complex

via EZH1 or EZH2. When present on the same histone tail, H3K4me3 and H3K36me3 are known to inhibit PRC2 complex activity and chromatin binding[117]. The switch from an actively transcribed to a repressive transcriptional state during differentiation is now known to be in part mediated by PHD finger protein 19 (PHF19), another H3K36me3 reader[118,119]. PHF19 has been shown to associate with the PRC2 complex to facilitate recruitment to H3K36me3 marked regions[119,120]. Interestingly, PHF19 also associates with the H3K36me3 demethylase NO66[118]. The opposing activities of SETD2 and NO66 mediate transitions between H3K36me3 and H3K27me3 modification[118]. Unsurprisingly, loss of SETD2 and H3K36me3 has been reported to result in increases in levels of H3K27me3 in gene bodies[121].

DNA methylation.: DNA methylation, another important epigenetic regulatory mark, involves the covalent addition of a methyl group to the 5'-position of cytosine residues, and usually occurs in the context of CpG dinucleotides. Although the maintenance of DNA methylation through replication is ensured by DNA (cytosine-5)-methyltransferase 1 (DNMT1), the establishment of de novo DNA methylation is mediated by DNA (cytosine-5)-methyltransferase 3A (DNMT3A) and DNA (cytosine-5)-methyltransferase 3B (DNMT3B)[122]. DNA methylation can occur at promoters, as well as in intergenic regions, gene bodies and enhancers. Promoter DNA methylation is considered to be a mark of transcriptional repression, whereas the function of gene body DNA methylation has been more difficult to elucidate and is generally associated with a permissive transcriptional state. H3K36me3 has been shown to mediate gene body DNA methylation through interaction with DNMT3B[123,124] (FIG. 4A).

Although both are capable of de novo DNA methylation, the genomic regions targeted by DNMT3A and DNMT3B differ. DNMT3A has been shown to preferentially methylate promoters and enhancers[125], whereas DNMT3B-bound genomic loci were specifically enriched for features of active transcription. DNMT3B-mediated DNA methylation occurs predominately in the linker regions between nucleosomes because of occlusion from the core nucleosome (FIG. 4A). Interestingly, DNMT3B-mediated DNA methylation occurs at the introns of genes marked with H3K36me3, but not those lacking H3K36me3[123]. Moreover, loss of H3K36me3 resulted in diminished DNMT3B–nucleosome binding and reduced gene body DNA methylation. Genomic regions bound by DNMT3B showed a marked enrichment for several transcription factor binding motifs containing CpG sequences, including motifs related to transcription factor SP1 and members of the ETS family. Both of these transcription factors have been reported to recruit the transcription machinery in TATA-less promoters, and their binding to DNA has been reported to be negatively affected by CpG methylation[126,127]. Thus, gene body DNA methylation, in a H3K36me3-dependent manner, might reduce the occurrence of cryptic transcription by preventing the aberrant binding of transcription factors within the gene body, and/or facilitate gene expression in a dynamic environment.

Altered DNA methylation patterns in RCC.: Alterations in DNA methylation patterns has been described in SETD2-deficient RCC. However, discrepancies exist regarding whether SETD2 loss results in DNA hypomethylation or hypermethylation[15,128–130]. These

discrepancies can be accounted for by differences in how the studies were conducted, which provides different clues into the complex crosstalk between epigenetic marks.

One study reported that SETD2 loss caused increased chromatin accessibility, characterized by nucleosome depletion, which substantially overlapped with gene bodies in RCC tumour specimens[15]. The authors subsequently assessed DNA methylation patterns in nucleosome-depleted regions associated with SETD2 loss using data from The Cancer Genome Atlas (TCGA) and predominantly observed DNA hypomethylation. These results suggest that SETD2 loss causes H3K36me3 depletion, and that those regions that lost H3K36me3 displayed increased chromatin accessibility and nucleosome depletion, and DNA hypomethylation. These findings are consistent with reports that H3K36me3 mediates DNMT3B-dependent DNA methylation[123].

Other studies have reported that SETD2 loss is associated with DNA hypermethylation. A comparison of DNA methylation between *SETD2* mutant and wild-type tumours from the ccRCC, pRCC, and chRCC TCGA datasets revealed that most changes involved increased global DNA methylation regardless of subtype[128]. The analysis was not restricted to any particular genomic location, and included promoters, gene bodies, enhancers or intragenic regions. Notably, the 450K DNA methylation assay (Illumina) that was used for the TCGA is enriched in promoter regions, thus ascertainment bias must be considered in interpreting these findings. Furthermore, *SETD2* mutations were enriched in pRCC tumour samples that had a CpG island methylator phenotype (CIMP)[128]. CIMP tumours are characterized by global DNA hypermethylation, which tends to occur CpG islands and are generally associated with promoter regions. This CIMP phenotype could skew the differential DNA methylation pattern in the *SETD2* mutant group towards DNA hypermethylation.

Another study noted that SETD2 loss caused increased DNA methylation in genes that are expressed at low levels, whereas highly expressed genes did not show changes in DNA methylation[129]. Other factors that govern the transcription of highly expressed genes, such as housekeeping genes, could possibly prevent aberrant DNA methylation from occurring. The authors also binned the probes on the 450K platform to identify differentially methylated regions (DMRs), which overlapped with regions that gained the H3K36me3 mark after SETD2 loss[129]; however, scaling presents a challenge in these studies in the absence of spike-in DNA references.

The examination of DNA methylation patterns using TCGA data identified three subclusters of RCC tumours that respectively showed no, low or high CIMP[130]. The CIMP-high group was associated with a poor prognosis and was enriched for *BAP1* and *SETD2* mutations. For the unsupervised clustering analysis that identified these three subclusters, the authors filtered the data to include only probes that displayed >20% methylation in any of the TCGA normal kidney samples (n=161)[130]. As noted by the authors, the resulting probes localized to promoter CpG islands. Thus, this analysis could be biased towards DNA hypermethylation, given that the probes included were selected so that they displayed hypomethylation in normal kidney samples (and presumably hypermethylation in tumours) and so that these probes were targeted to promoter CpG islands, which typically undergo DNA hypermethylation.

Noncanonical functions in RCC

Evidence indicates that chromatin modifiers have nonhistone substrates and, in some cases, even travel outside the nucleus. These findings suggest that these enzymes have noncanonical functions, including roles in metabolic control, cytoskeletal remodeling and interferon signalling, and reports suggest that these noncanonical functions, when lost, also contribute to RCC[131–133].

Extranuclear roles of BAP1—Under normal growth conditions, BAP1 is predominantly localized in the nucleus. However, BAP1 can be ubiquitylated by ubiquitin-conjugating enzyme E2 O (UBE2O) at its NLS to promote nuclear export[134]. UBE2O-mediated ubiquitylation causes BAP1 to accumulate in the cytoplasm, whereas BAP1 autodeubiquitylation promotes nuclear retention. Although BAP1 localization and function is best characterized in the nucleus, it is conceivable that BAP1 could have extranuclear functions. Indeed, experimental evidence that BAP1 loss alters cellular metabolism — which primarily occurs in the cytoplasm and in mitochondria — supports this possibility.

BAP1$^{+/-}$ fibroblasts derived from patients with RCC with germline *BAP1* mutations had an altered metabolism that was characteristic of the Warburg effect, as shown by increased glycolysis and reduced mitochondrial respiration[135]. In RCC cells, BAP1 inhibited glucose deprivation-induced apoptosis through interaction with the activating transcription factor 3 (*ATF3*) and DNA damage-inducible transcript 3 protein (*DDIT3*) gene promoters in a polycomb complex protein BMI-1-dependent manner[136]. Loss of BAP1 increased the production of reactive oxygen species (ROS) and activated endoplasmic reticium (ER) stress pathways. In addition, loss of BAP1 has also been described to deplete mitochondrial proteins in pancreas cells[137]. This observation is in contrast to a report in mesothelioma cells, in which BAP1 loss caused increased mitochondrial mass[138]. Notably, although BAP1 loss is associated with poor prognosis in patients with ccRCC, its loss is a favourable prognostic marker in mesothelioma[139]. Collectively, these evidences suggest that the metabolic effects of BAP1 loss could be cell-type specific.

Although the exact mechanism underpinning the metabolic alterations associated with BAP1 loss are currently unknown, a study from 2017 reported that BAP1 promotes calcium (Ca^{2+}) release from the ER to the mitochondria[135]. This process was shown to be dependent on BAP1-mediated deubiquitylation of type 3 inositol-1,4,5-trisphosphate receptor (IP3R3), an ER channel that regulates Ca^{2+} release from the ER to the cytoplasm and mitochondria. Transient Ca^{2+} release leads to mitochondrial ATP production, whereas excessive or prolonged Ca^{2+} release promotes apoptosis via excessive mitochondrial Ca^{2+} levels and the consequent opening of the mitochondrial permeability transition pore[140]. The authors suggest that malignancies that are most frequently associated with the BAP1 cancer syndrome, defined by the germline loss of function of one allele of *BAP1* (which predisposes to ccRCC, uveal and cutaneous melanoma, mesothelioma, and melanocytic BAP1-mutated atypical intradermal tumours), arise from cells or tissues in which Ca^{2+}-induced apoptosis is crucial for preventing cellular transformation[141].

SETD2 in microtubule midbody assembly—Historically, SETD2 was presumed to only act upon lysine 36 on histone H3. However, a study from 2016 reported that SETD2 could also trimethylate α-tubulin on lysine 40 (TubK40me3)[131]. The heterodimerization of α-tubulin with β tubulin gives rise to long, dynamic polymeric molecules called microtubules, and the acetylation of lysine residues on alpha tubulin is known to be involved in the polymerization process[142]. Alpha tubulin methylation and acetylation at lysine 40 were shown to be reciprocal marks that compete for the same lysine residue. Loss of both TubK40me3 and H3K36me3 was observed in mouse embryonic fibroblasts (MEFs) with conditional knockout of *Setd2*[131]. These *SetD2*-knockout MEFs displayed marked genomic instability and had mitotic and cytokinetic defects, including increased micronuclei, failure of chromosome congression and increased multinuclear spindles during prometaphase, lagging chromosomes during anaphase and chromosomal bridges during cytokinesis[131]. SETD2 loss was also shown to promote micronuclei formation in ccRCC[133]. This novel function of SETD2 in catalyzing TubK40me3 sheds light on how SETD2 loss contributes to cellular transformation and aggressiveness.

BAP1 in cytoskeleton remodeling—BAP1 has been reported to deubiquitylate γ-tubulin at lysine 48 and lysine 344[143]. In eukaryotes, the function of γ-tubulin is highly conserved and includes the regulation of microtubule nucleation and centrosome duplication[144]. Ubiquitination of γ-tubulin at lysine 48 and lysine 344 is mediated by the activity of the tumour suppressive complex breast cancer susceptibility gene 1 (BRCA1)–BRCA1-associated RING domain protein 1 (BARD1), a complex that includes BAP1[145]. Interaction between BAP1 and γ-tubulin occurs during metaphase, anaphase and telophase, but not during prophase, during which γ-tubulin is ubiquitylated. BAP1 was found to be necessary for proper mitotic spindle organization and prevention of chromosomal abnormalities, which include lagging chromosomes during metaphase, uncondensed mitotic chromosomes, anaphase and telophase bridges and tripolar mitotic spindles[143].

In RCC cells, BAP1 has also been shown to have a role in mediating chromosome segregation by regulating the stability of microspherule protein 1 (MCRS1) — which is involved in various biological processes, such as transcription, proliferation, mitosis and senescence[146] — through de-ubiquitylation, preventing its proteasomal degradation in RCC cells[147]. Knockout of *MCRS1* resulted in chromosome instability and aneuploidy in mammary cells[146]. Similarly, BAP1 depletion induced multipolar spindle formation and multinucleation, which were partially rescued by the stable overexpression of MCRS1 in RCC cells[147]. These findings collectively suggest that BAP1 maintains chromosomal stability by modulating the ubiquitylation status of various substrates, including MCRS1 and γ-tubulin.

SETD2 and PBRM1 in interferon signalling—SETD2 has been reported to mediate signal transducer and activator of transcription 1 (STAT1) methylation on lysine 525[132], which facilitates STAT1 phosphorylation by Janus kinase 1 (JAK1) and the consequent activation of STAT1 as a transcription factor to mediate transcriptional responses to interferon signals. SETD2 loss-of-function resulted in impaired STAT1 signalling,dampening the host response to Hepatitis C virus infection in human

hepatocytes[132]. This finding further emphasizes the potential importance of the nonhistone substrates and noncanonical functions of SETD2. The impact of this activity of SETD2 in RCC remains to be determined.

The SWI/SNF complex has previously been described to have a role in regulating host defense against viral infection. Loss of SMARCB1 was shown to block cellular response to viral infection and impaired antiviral activity by inhibiting expression of virus-inducible and IFN-inducible genes in HeLa cells[148]. Similarly, *PBRM1* knockdown in the ACHN RCC cell line altered the cytokine–cytokine receptor interaction pathway, characterized by upregulation of interleukin-6 receptor subunit beta (IL6ST) and C-C motif chemokine 2 (CCL2) and downregulation of IL-8, IL-6, and C-X-C motif chemokine 2 (CXCL2)[149]. These studies suggest that dysfunction of the SWI/SNF complex through loss-of-function of the PBRM1 and SMARCB1 subunits impairs cellular antiviral responses.

Conversely, other studies have shown that knockdown of *PBRM1* results in unrestrained cellular antiviral responses and increased tumour immunogenicity. *PBRM1* knockdown in colon cancer cells caused a robust upregulation of retinoic acid-inducible gene 1 protein (RIG-I)-like receptor signalling genes, such as viral RNA receptors (*RIGI* and *MDA5*) and their downstream transcriptional targets (*IRF7*, *ISG15*, *ISG56*, and *IRF9*)[49]. In *Drosophila*, PBRM1-deficient intestinal cells were susceptible to infection as a result of hyper-inflammation[150]. PBRM1 was shown to cooperate with EZH2 to directly suppress RIG-I and MDA5 transcription, and therefore, knockdown of *PBRM1* resulted in unrestrained transcription of target genes[49]. In this regard, gene expression analyses in PBRM1-deficient and PBRM1-proficient RCC cells showed that PBRM1 deficiency induced expression of a number of genes related to immune response, such as those involved in IL-6–Janus kinase (JAK)–signal transducer and activator of transcription 3 (STAT3) signalling, TNF signalling via nuclear factor-κB (NF-κB), and IL-2–STAT5 signalling, as well as genes involved in hypoxia signalling[48]. These conflicting reports highlight the complexity of SWI/SNF complex-mediated regulation of viral defense genes and suggest that the effect that loss of SWI/SNF complex function has on host viral defense is dependent on which subunit is lost, as well as the cell type in which the subunit is lost. The relevance of PBRM1 loss in RCC and its effect on response to therapy is now beginning to be understood.

Therapeutic opportunities

In contrast to gain-of-function mutations in oncogenes, which are conceptually straightforward to target, loss-of-function mutations in tumour suppressor genes are more challenging to approach therapeutically. Synthetic lethality describes a relationship between two genes whereby loss of both genes is incompatible with cell survival, whereas loss of either gene does not induce cell death. Thus, by exploiting the phenomenon of synthetic lethality, loss-of-function mutations in chromatin modifiers could present unique therapeutic opportunities in RCC.

Loss of SETD2—Loss of the yeast SETD2 homolog Set2 was shown to be synthetically lethal with loss of mitosis inhibitor protein kinase Wee1 in *Schizosaccharomyces pombe*[151]. This synthetic lethality was also observed when the human RCC cell lines A498 and

LB996 — which harbour naturally-occurring *SETD2* mutations — were treated with MK1775, a selective inhibitor of the human Wee1 homolog Wee1-like protein kinase (WEE1)[151]. WEE1 is a serine/tyrosine protein kinase that has a crucial role in enabling DNA repair to occur before mitotic entry during the G2–M cell cycle checkpoint arrest. Normal cells repair damaged DNA during G1 arrest; however, cancer cells usually have a deficient G1–S checkpoint and, therefore, depend on a functional G2–M checkpoint to repair DNA damage[152]. Indeed, SETD2-deficient osteosarcoma cells treated with a WEE1 inhibitor showed signs of replicative stress, replication fork stalling and S phase arrest[151]. Ribonucleoside-diphosphate reductase subunit M2 (RRM2), a component of ribonucleotide reductase (RNR), was shown to mediate this synthetic lethality through two mechanisms in RCC as well as osteosarcoma cells. First, SETD2-mediated H3K36me3 was found to be necessary for *RRM2* transcription, and loss of H3K36me3 reduced *RRM2* transcription[151]. Second, WEE1 inhibition was found to mediate RRM2 degradation through activation of cyclin-dependent kinase 1 (CDK1) and/or CDK2[151]. In osteosarcoma cells, WEE1 inhibition has also been reported to cause aberrant origin firing through CDK activation, which, similar to RRM2 depletion, exhausts the deoxyribonucleotide triphosphate (dNTP) pool[153]. Aberrant origin firing, synergized with RRM2 depletion, was found to cause synthetic lethality in RCC cells[151]. These findings emphasize the importance of SETD2 in DNA repair and imply that exploiting this synthetic interaction could lead to effective therapies in at least selected cellular backgrounds.

Using the publicly available Genomics of Drug Sensitivity in Cancer (GDSC) database, TGX221, a selective phosphoinositide 3-kinase β (PI3Kβ) inhibitor, was identified as a potential drug candidate in ccRCC tumours with *VHL* and *SETD2* mutations[105]. In vitro experiments demonstrated that ccRCC cells with mutations in both *VHL* and *SETD2* were sensitive to TGX221. However, sensitivity to TGX221 was not observed in SETD2-competent ccRCC cells harbouring *VHL*-inactivating mutations. SETD2-deficient cells have also shown sensitivity to another PI3Kβ inhibitor, AZD6482[154]. Inhibition of PI3Kβ in *SETD2*-mutant ccRCC cells led to decreased phosphorylation of protein kinase B (AKT) at both Serine 473 and Threonine 308, which was not accompanied by a change in total AKT levels[155]. Interestingly, loss of MutL protein homolog 1 (MLH1) conferred resistance to PI3Kβ inhibitors in *SETD2*-mutant ccRCC cells, and analysis of the TCGA database revealed a high tendency of concurrent homozygous loss of *SETD2* and *MLH1*[155]. Further investigation showed that SETD2 also mediates MMR via activation of AKT and repression of *MLH1* transcription to regulate the expression of the mismatch repair endonuclease PMS2, an essential component of the MMR response. The authors speculate that SETD2 indirectly activates AKT via the JAK–STAT pathway. Indeed, the aforementioned report that SETD2 mediates STAT1 methylation on lysine 525 supports this theory[132]. These findings further emphasize the potential importance of the nonhistone substrates and noncanonical functions of SETD2 in normal cells, as well as in *SETD2*-deficient RCC cells.

Loss of BAP1—Loss of *BAP1* has been shown to be synthetically lethal with inhibition of EZH2 or PRC2. In a mouse model, *Bap1*-knockout induced myeloid malignancy and resulted in increased H3K27me3 levels, elevated EZH2 protein expression and enhanced repression of PRC2 targets[156]. To further assess the role of PCR2-mediated H3K27me3

in cellular transformation mediated by *Bap1* loss, the authors investigated the influence of *Ezh2* loss on transformation in vivo. *Ezh2* loss in *Bap1*-knockout mice resulted in decreased levels of H3K27me3 and abrogated the development of myeloid malignancy. Moreover, treatment of *Bap1*-knockout mice with EPZ011989, a small molecule inhibitor of EZH2, recapitulated the in vivo-effects observed with knockout of *Ezh2*[156]. In BAP1-deficient human mesothelioma cells, EZH2 inhibition also produced synthetic lethality[156]. However, this synthetic lethality between BAP1 and EZH2 was not observed in uveal melanoma cell lines[157,158], suggesting cell-type specificity. Thus, studies are warranted to determine whether BAP1-deficient RCC cells display sensitivity to EZH2 inhibition.

Loss of SWI/SNF complex components—Synthetic lethality between some components of the SWI/SNF complex (PBRM1, SMARCB1, BRG1 and ARID1A) and EZH2 has also been reported in RCC[159,160], as well as in other cancers[159–161]. This synthetically lethal relationship between SWI/SNF complex components and EZH2 was shown to be mitigated by mutations in members of the RAS–mitogen activated protein kinase (MAPK) pathway[159]. Preclinical studies have shown that PBRM1-deficient RCC cells and SMARCB1-deficient malignant rhabdoid tumour cells are sensitive to EZH2 inhibitors[159,160]. Interestingly, PBRM1 exhibited a synthetically lethal interaction with the H4K16ac acetyltansferase KAT5 in osteosarcoma cells[162]. ARID1A-deficient breast, ovarian, lung and colorectal cancer cells also showed sensitivity to PI3K–AKT pathway and PARP1 inhibitors[163,164]. However, monotherapy with PI3K–AKT–mechanistic target of rapamycin (mTOR) pathway inhibitors has shown limited clinical efficacy in RCC, suggesting that combinational therapies could be necessary[163,165].

These evidences demonstrate that deciphering the downstream oncogenic sequelae of mutations in chromatin modifiers is crucial to the rational and hypothesis-driven development of novel therapeutics for RCC. In addition, the influence of other co-drivers is also important in determining response to therapy. Thus, the identification of mutations in chromatin modifer genes in RCC tumour samples could become important in the clinical management of patients with RCC.

Loss of PBRM1 and immunotherapy—Although immunotherapies have gained considerable attention, the mechanisms governing their efficacy remain poorly understood. The efficacy of immunotherapies is thought to lie in the ability of cytotoxic T cells to detect and eliminate transformed cells following recognition of peptide antigens displayed by major histocompatibility (MHC) class I proteins[166]. Interestingly, components of the PBAF complex, specifically *Arid2*, *Brd7* and *Pbrm1*, were identified in a CRISPR–Cas9 functional genomic screen as tumour cell-intrinsic genes that conferred resistance to T cell-mediated killing of B16F1 mouse melanoma cells[50]. Genes involved in the NF-κB pathway, mTOR complex 1 (mTORC1) signalling, glycolysis, and nicotinate and nicotinamide metabolism, were also identified toconfer resistance to T cell-mediated B16F10 killing[50]. T cell-mediated killing of B16F10 melanoma cells was enhanced by loss of *Arid2*, *Brd7* or *Pbrm1*. *Pbrm1* loss in B16F10 cells conferred therapeutic benefit to anti-programmed cell death protein 1 (PD-1) and/or anti-cytotoxic T-lymphocyte protein 4 (CTLA-4) immune checkpoint blockade, whereas this treatment was ineffective in control B16F10 cells, which are resistant

to these therapies[50]. In another study, patients with metastatic RCC harbouring truncating PBRM1 mutations derived more clinical benefit from immune checkpoint blockade than patients with PBRM1 wild-type tumours[48]. The authors suggested that the increased clinical benefit in patients with PBRM1 truncating mutations was due to the unique immune-related gene expression signature observed in PBRM1-deficient RCC cells[48]. These findings, together with evidence supporting the role of PBRM1 in regulating host antiviral response, suggest that PBRM1 loss promotes RCC immunogenicity through hyperactivation of interferon-responsive genes and causes RCC cells to be 'visible' to the immune system, which could in turn confer sensitivity to immune checkpoint blockade.

Conclusions

Loss-of-function of chromatin modifiers, including PBRM1, BAP1 and SETD2, among others, is prevalent in RCC, and these events have long been considered as important co-drivers in this disease. These chromatin modifiers have far-reaching, and sometimes unexpected, functions. Classically, chromatin modifiers are known for their canonical functions, which include roles in DNA repair and transcriptional regulation. In recent years, histone modifiers have been found have nonhistone substrates and engage in noncanonical activities, such as the regulation of cytoskeletal dynamics and interferon signalling. Although canonical functions are undoubtedly important, loss of these noncanonical functions more than likely also contributes to RCC tumorigenesis and progression.

Mounting evidence indicates that clinical behaviour and response to treatment modalities can be influenced by the particular repertoire of mutations in chromatin modifiers. Furthermore, loss-of-function mutations in these chromatin modifiers might create unique therapeutic opportunities, such as those afforded by exploiting synthetic lethal dependencies. Accordingly, there is renewed urgency to further understand their etiology and how loss-of-function of these chromatin modifiers contributes to RCC tumourigenesis and progression. This knowledge will not only be pivotal for the rational and hypothesis-driven development of novel therapeutic strategies in RCC, but will also be essential as we usher in the age of personalized medicine.

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Key points

- Loss-of-function mutations in chromatin modifiers, common in renal cell carcinoma (RCC), can modify tumour biology and influence therapeutic responses; thus, understanding how these events contribute to RCC is paramount.
- Chromatin modifiers classically regulate genomic architecture and, therefore, control DNA accessibility; these canonical functions are fundamental for essential cellular processes, such as gene expression programs and DNA damage repair.
- Chromatin modifiers have non-histone substrates and participate in extranuclear processes; these noncanonical functions regulate important cellular processes such as cytoskeletal dynamics and immune responses.
- Loss-of-function mutations in chromatin modifiers can be approached therapeutically by exploiting synthetically lethal dependencies between two genes; loss of both genes induces cell death, but loss of either is nonfatal.

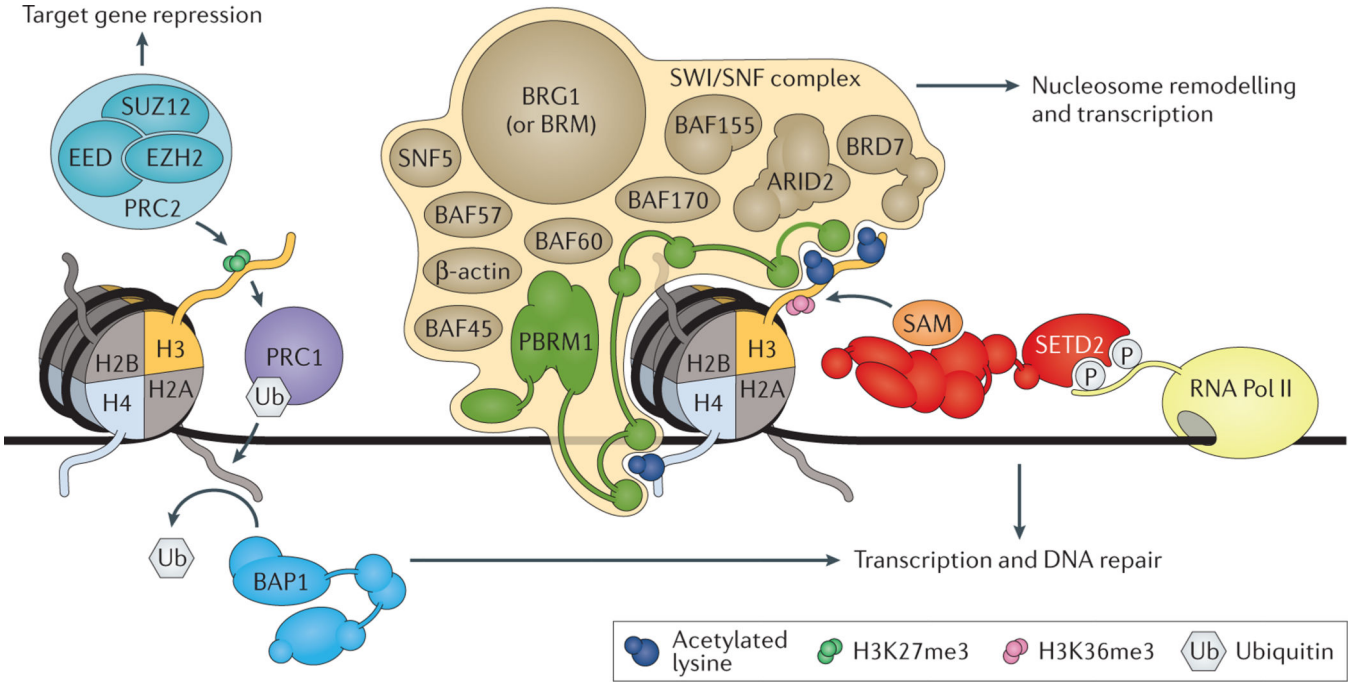

Figure 1 |. Chromatin modification by PBRM1, BAP1 and SETD2.

The switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling complex, shown here as polybromo-associate BAF (PBAF), is comprised of >11 subunits, including: a catalytic core subunit (protein brahma homolog (BRM) or transcription activator BRG1); accessory subunits (β-actin, SNF5, BAF45, BAF57, BAF60, BAF155, and BAF170); and DNA binding subunits (AT-rich interactive domain-containing protein 2 (ARID2), bromodomain-containing protein 7 (BRD7), and protein polybromo-1 (PBRM1); or alternatively, ARID1A and ARID1B). PBRM1 contains 6 bromodomains that mediate DNA targeting by binding acetylated histone residues. Histone-lysine N-methyltransferase SETD2 associates with hyperphosphorylated RNA polymerase II (RNA Pol II) and deposits the histone H3 lysine 36 trimethyl mark (H3K36me3) as it travels along with RNA Pol II during transcription; SETD2 uses S-Adenosyl methionine (SAM) as a one carbon donor during methylation. Consequently, SETD2 deposits the H3K36me3 mark primarily on exons of actively transcribed genes. Thus, SETD2 and its associated mark (H3K36me3) are involved in regulating transcription and mediating DNA damage repair. Polycomb repressive complex 1 (PRC1) and PRC2 are responsible for regulating cellular differentiation and lineage commitment and maintenance via transcriptional repression of target genes. PRC2, comprised of polycomb protein SUZ12, polycomb protein EED, and histone-lysine N-methyltransferases EZH1 or EZH2, catalyzes the addition of the trimethyl mark on lysine 27 of histone H3 (H3K27me3), a mark that is associated with gene silencing. Canonically, H3K27me3 subsequently recruits PRC1 to reinforce gene repression through mono-ubiquitylation of lysine 119 on histone H2A (H2AK119ub1). PRC1 activity can be reversed by ubiquitin carboxyl-terminal hydrolase BAP1, a protein deubiquitinase that catalyzes the deubiquitination of H2AK119ub1. BAP1-mediated deubiquitination of H2AK119ub1 has important roles in regulating transcription and mediating DNA damage repair.

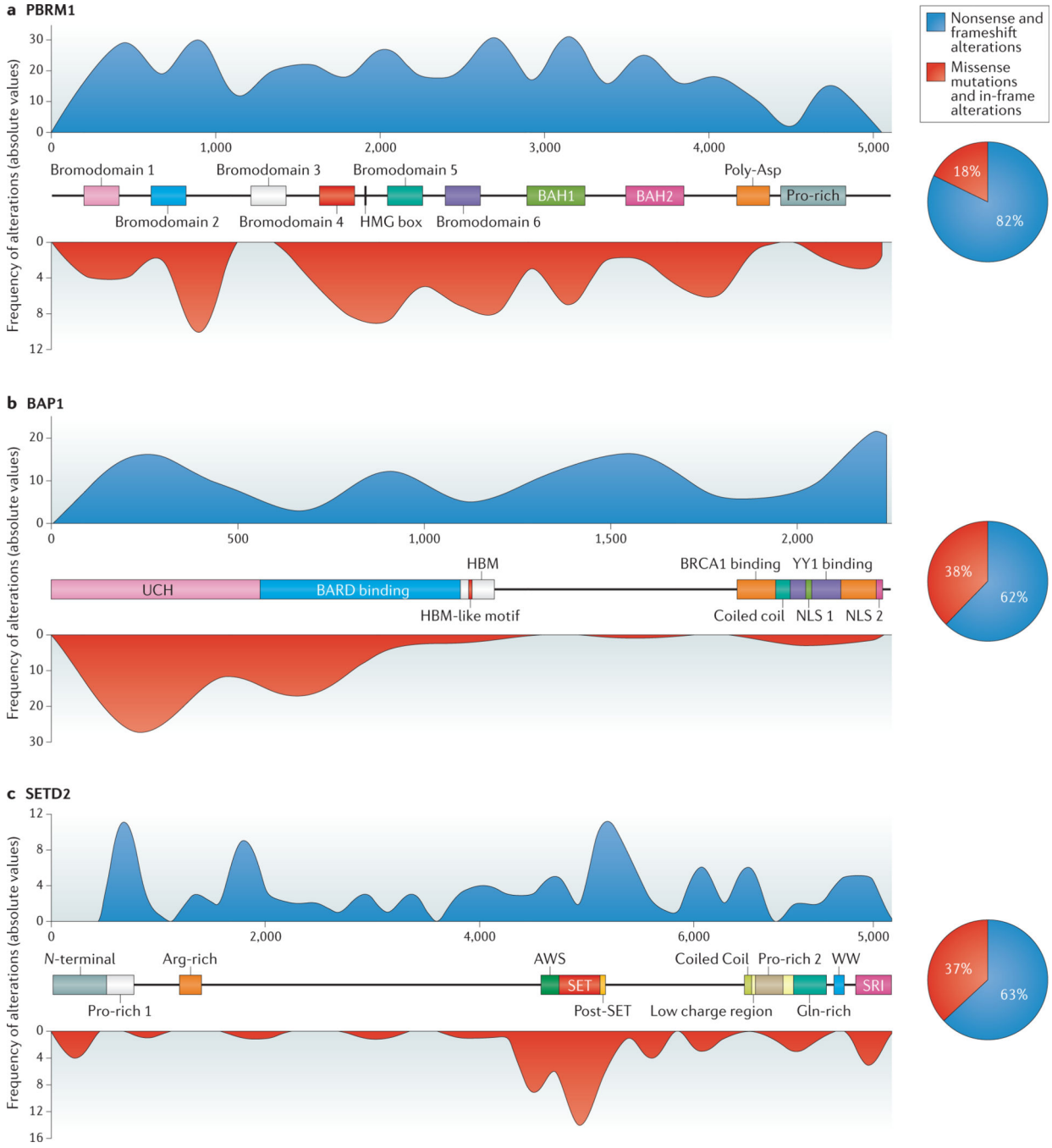

Figure 2 |. Structure and distribution of PBRM1, BAP1 and SETD2 mutations in RCC.

The known protein domains and motifs are indicated for protein polybromo-1 (PBRM1; also known as BAF180) (part **a**), ubiquitin carboxyl-terminal hydrolase BAP1 (part **b**) and histone-lysine N-methyltransferase SETD2 (part **c**). The overlaid blue (nonsense and frameshift alterations) and red (missense mutations and in-frame alterations) histograms represent the distribution of genetic alterations, previously reported in RCC, along each gene[40,41]. The X axis represents the position of cDNA bases and the Y axes for each respective histogram shows the frequency of alterations (absolute values). The associated pie

charts show the proportion of nonsense and frameshift alterations (blue area) and missense and in-frame alterations (red area). Data from REFs [40,41].BAH, bromo-adjacent homology; HMG, high-mobility group; UCH, ubiquitin C-terminal hydrolase; HBM, HCF-C1 binding motif; SRI, SET2-Rpb1 interacting; NLS, nuclear localization signal.

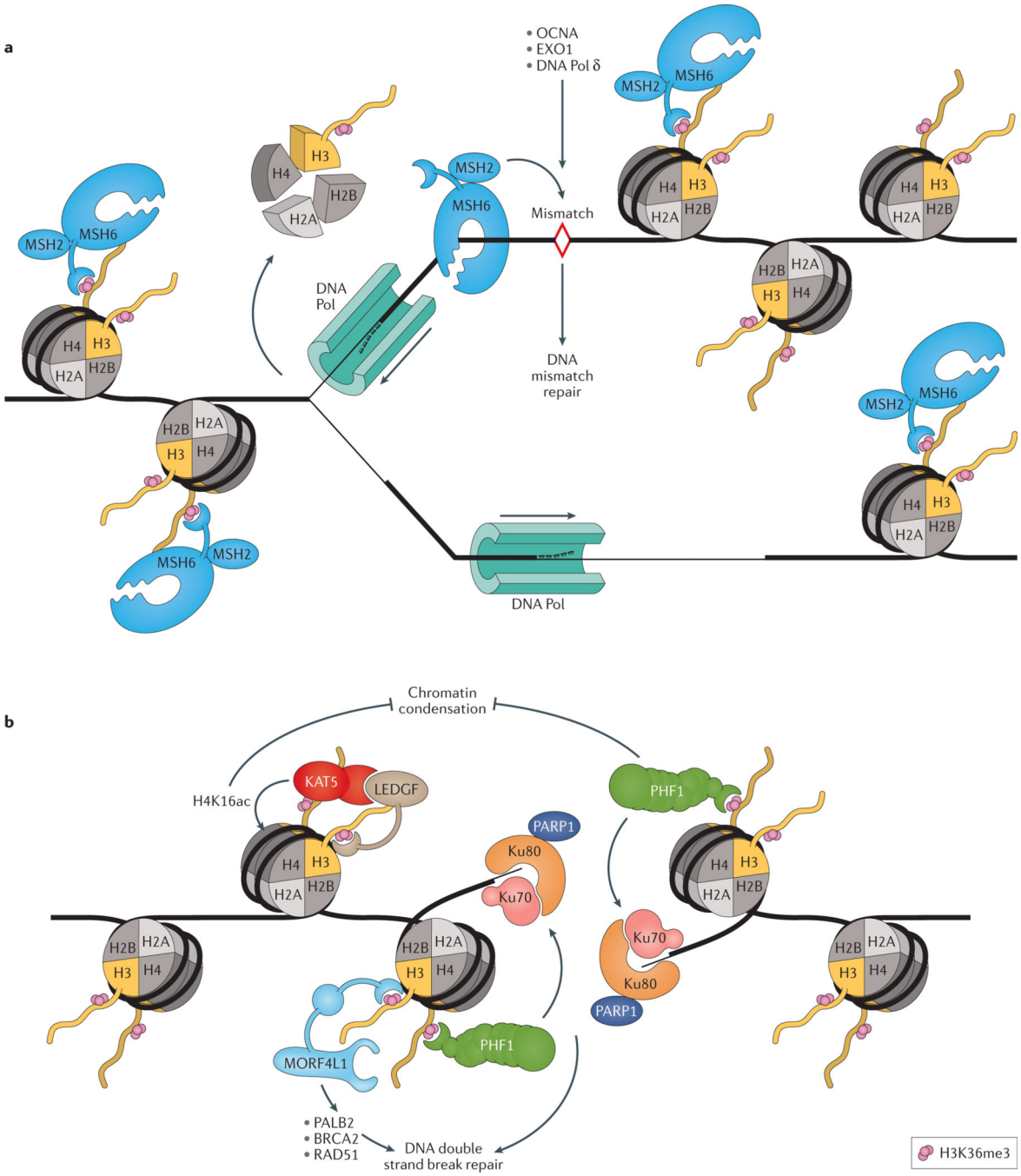

Figure 3 |. H3K36me3 facilitates DNA damage repair.

a | During DNA replication, misincorporated nucleotides and insertion–deletion mispairs are repaired through DNA mismatch repair (MMR). These replication errors can be recognized by the MutSα complexes (composed of a MSH2-MSH6 heterodimer), which can initiate MMR by recruiting proliferating cell nuclear antigen (PCNA), exonuclease 1 (EXO1) and DNA polymerase δ (DNA Pol δ) to sites of DNA damage. MutSα is recruited to genomic regions decorated with trimethylated histone H3 lysine 36 (H3K36me3), via the PWWP domain of MutS protein homolog 6 (MSH6). This nuance guarantees that

MutSα is present and enriched for in coding sequences prior to DNA replication and ensures that MMR is concentrated at these regions. **b |** H3K36me3 also has a role in the repair of DNA double strand breaks (DSBs). When DSBs occur, H3K36me3 marks on nearby nucleosomes becomes exposed, leading to the recruitment of PHD finger protein 1 (PHF1) to the DSB site, where it locally recruits X-ray repair cross-complementing protein 6 (Ku70)–X-ray repair cross-complementing protein 5 (Ku80) complexes and PARP1 to promote the initiation of DSB repair. Mortality factor 4-like protein 1 (MORF4L1; also known as MRG15) also participates in DSB repair by recruiting partner and localizer of BRCA2 (PALB2), which then recruits breast cancer type 2 susceptibility protein (BRCA2) and DNA repair protein RAD51 homolog 1 (RAD51) to DSB sites. Maintaining an open chromatin state creates a favourable environment conducive to the resolution of DSBs. PHF1 also facilitates DSB repair by inhibiting the activity of factors involved in chromatin condensation, promoting a more relaxed, open chromatin state. Following DNA DSBs, MORF4L1 recruits lens epithelium-derived growth factor (LEDGF; also known as PSIP1), which subsequently recruits KAT5, a histone H4 lysine 16 (H4K16ac) acetyltransferase, and increases H4K16ac, which prevents chromatin condensation.

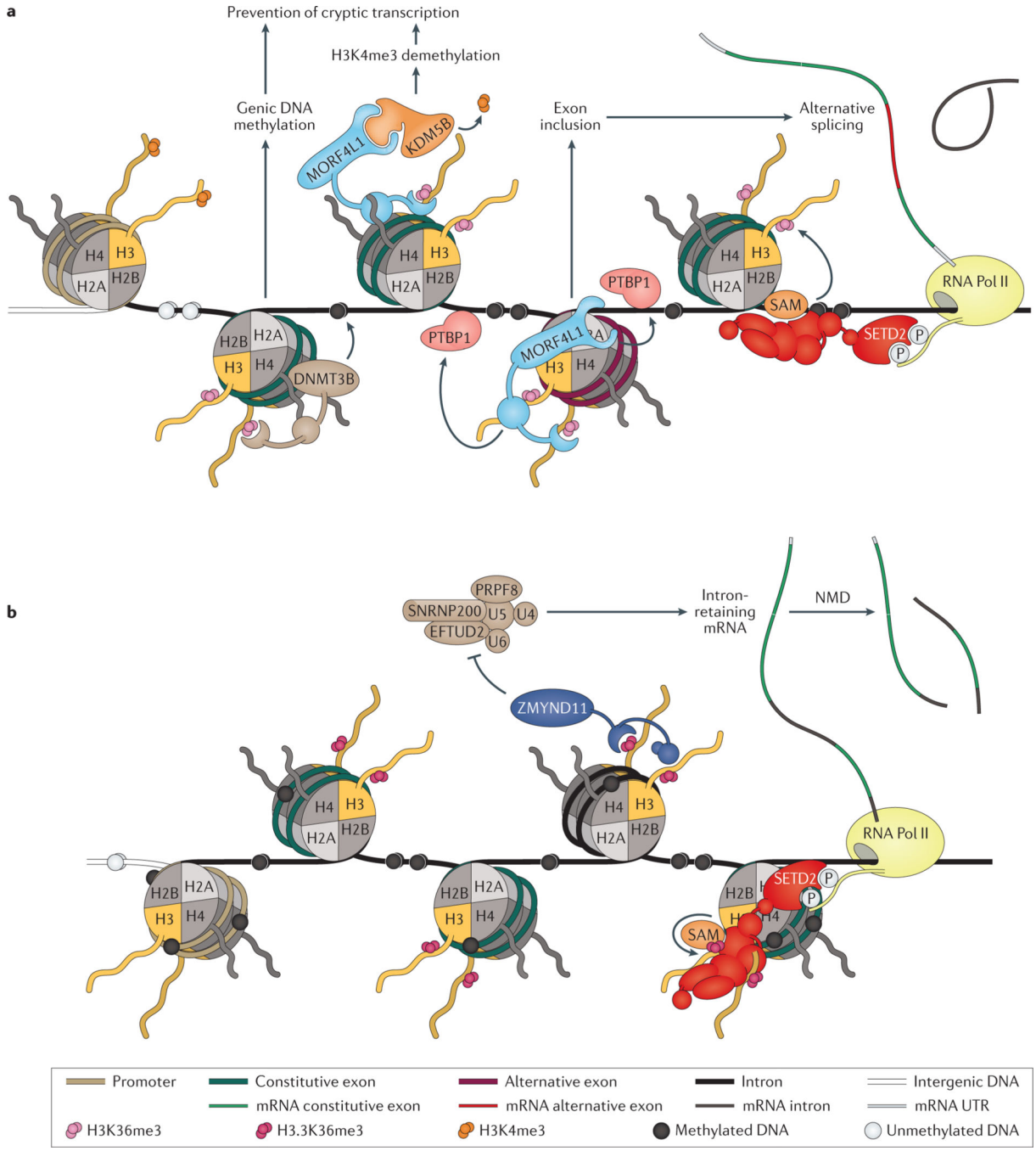

Figure 4 |. Maintenance of transcriptional fidelity.

a | DNA (cytosine-5)-methyltransferase 3B (DNMT3B) mediates gene body DNA methylation through its interaction with trimethylated lysine 36 on histone H3 (H3K36me3) via its PWWP domain. DNMT3B can prevent spurious transcriptional initiation by promoting the DNA methylation of intragenic transcription factor binding motifs, which can influence the binding of some transcription factors. Mortality factor 4-like protein 1 (MORF4L1; also known as MRG15) also binds to H3K36me3 and can recruit lysine-specific demethylase 5B (KDM5B), the trimethylated lysine 4 on histone H3 (H3K4me3)

lysine demethylase. The activity of KDM5B prevents spurious transcription initiation by removing H3K4me3, which is associated with active gene promoters. In addition, MORF4L1 can mediate gene splicing, whereby MORF4L1 recruits polypyrimidine tract-binding protein 1 (PTBP1) to DNA to promote exon inclusion in the mature mRNA transcript. **b |** A histone H3K36me3-specific reader, zinc finger MYND domain-containing protein 11 (ZMYND11; also known as BS69), mediates intron retention of certain target genes. ZMYND11 contains a bromo-PWWP domain that specifically binds trimethylated lysine 36 on the histone H3.3 variant (encoded by the *H3F3A* or *H3F3B* genes), rather than the canonical histone H3.1 or H3.2. Incorporation of the H3.3 variant histone into chromatin is independent of DNA synthesis, and it is deposited at distinct genomic regions, including gene bodies, telomeres, and pericentric chromatin. Histone H3.3 phosphorylation at serine 31 abrogates ZMYND11 binding to H3.3K36me3, suggesting that ZMYND11 recognition of H3.3K36me3 is regulated by signalling pathways that mediate histone H3.3 serine 31 phosphorylation. ZMYND11 inhibits the activity of the U5 small nuclear ribonucleoprotein particle protein complex — which comprises U5 small nuclear ribonucleoprotein 40 kDa protein (U5), 116 kDa U5 small nuclear ribonucleoprotein component (EFTUD2), pre-mRNA-processing-splicing factor 8 (PRPF8), U5 small nuclear ribonucleoprotein 200 kDa helicase (SNRNP200), U4 (RNU4–1), and U6 (RNU6–1) — to promote intron retention, which can trigger the nonsense mediated decay (NMD) pathway. The NMD surveillance pathway usually degrades erroneous transcripts, which contain premature a stop codon, and in this way, can reduce gene expression and could regulate transcriptional output.