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"We expect that the fever-pitch progress in chromatim drug discovery will yield new agents that provide improved, efficacious cancer drugs"

Keynote review: Chromatin control and cancer-drug discovery: realizing the promise

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Recent years have seen major advances in elucidating the complexity of chromatin and its role as an epigenetic regulator of gene expression in eukaryotes. We now have a basic understanding of chromatin control and the enzymatic modifications that impart diverse regulatory cues to the functional activity of the genome. Most importantly, although research into chromatin has uncovered fascinating insights into the control of gene expression, it has also generated a large body of information that is being harnessed to develop new therapeutic modalities for treating cancer. Here, we discuss recent advances that support the contention that future generations of chromatin-modulating drugs will provide a significant group of new, mechanism-based therapeutics for cancer.

In eukaryotic cells, histone proteins assemble the vast amount of genomic DNA into a size and structure that can be accommodated easily by the nucleus. Core histones are small basic proteins that form a structure, known as the nucleosome, consisting of a histone octamer in which an H3–H4 tetramer and two H2A–H2B dimers wrap around ~146 bp of DNA [1]. Together with the linker histone H1, nucleosomes assemble into higher-order structures that allow further rounds of compaction and condensation to occur. In addition, several histone variants have specialized roles in, for example, the DNA-damage response in which the H2A variant, H2AX, localizes to areas of DNA damage [2]. Other proteins, such as high-mobility group (HMG), also contribute to chromatin structure [3,4].

In the early days of chromatin research, it was thought that nucleosomes provided a natural barrier to transcription factors that bind DNA and, therefore, nucleosomal DNA was thought to have low transcriptional activity. However, nucleosomes are remodelled by the action of chromatin-remodelling complexes, a family of ATP-dependent molecular machines that share a related ATPase subunit and change nucleosome positions and disrupt DNA–histone interactions [5,6]. In addition, it has become clear that histones have an extensive repertoire of posttranslational modifications, which emanate from a diverse set of chromatin-associated proteins that target mainly the charged, N-terminal-tail regions [7,8]. These covalent modifications, which include acetylation, methylation and phosphorylation, have dramatic influences on gene expression by altering chromatin accessibility and they provide scope for many additional

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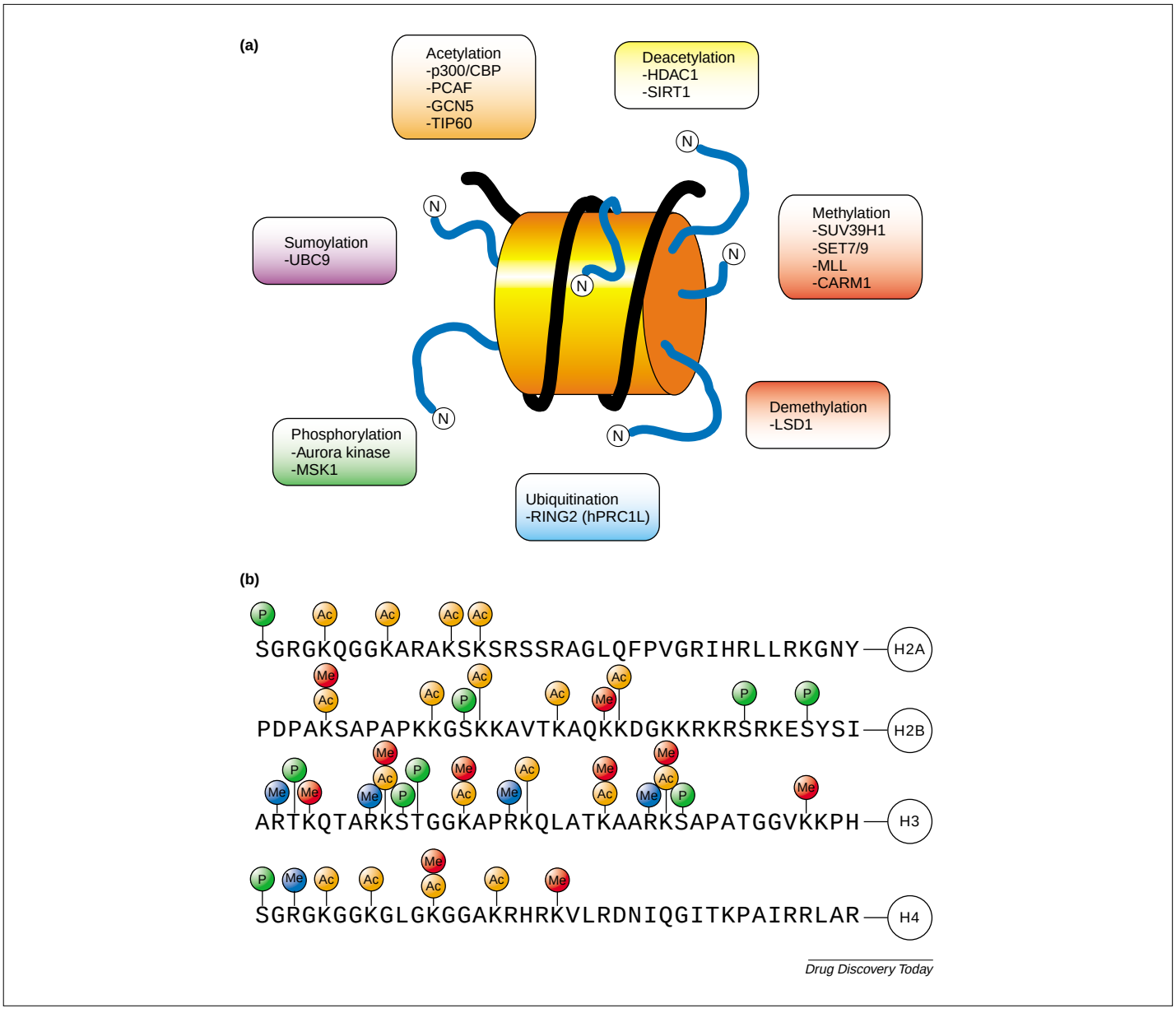

FIGURE 1

Enzymes involved in the modification of chromatin. (a) DNA (black line) wrapped around a nucleosome (orange cylinder), with histone tails and N-terminal regions in blue. Known posttranslational modifications and some of the enzymes responsible for these are given in boxes. Acetylation, methylation and phosphorylation are discussed in detail in this review and the roles of sumoylation and ubiquitination in chromatin control are reviewed in [160,161]. **(b)** Modifications and their locations on the tails of core histones, H2A, H2B, H3 and H4. Spheres indicate the residue that is modified and the type of modification; methylation of lysines (red), methylation of arginines (blue), acetylation of lysines (orange), phosphorylation of serine or threonine (green). Ubiquitination and sumoylation, which occur on lysine residues, are not included.

protein interactions [9]. Studies on the posttranslational modification of histones have given rise to the concept of the 'histone code'. This has been suggested to provide an additional layer of control that dictates the level of gene expression and it is mediated principally through regulating protein interactions. In combination with the DNA code, the histone code provides a signalling network of enormous potential complexity that allows gene expression to be finely tuned to the requirements of the cell.

Histone tails are modified by an extensive, expanding group of non-histone chromatin-associated proteins. These usually exist in cells as multicomponent protein complexes that are recruited frequently to chromatin in association with DNA-bound transcription factors [10,11]. Some of the proteins in these complexes have intrinsic enzyme activities that target discrete residues in histone tails with high precision. These proteins fall into several enzyme classes, including histone acetyltransferases (HATs) and the opposing enzyme activity, histone deacetylase (HDAC), methyltransferases (targeting both lysine and arginine residues) and protein kinases (Figure 1).

If we consider the histone-tail regions, especially from H3 and H4, many modifications are possible (Figure 1b), and it is the precise constellation of these modifications, namely the histone code, that dictates the final 'readout' of transcriptional activity. This is mediated, in part, through the ability of protein domains that are

embedded in chromatin-associated proteins to recognize modified histone residues, thereby allowing the recruitment of multicomponent–protein complexes that influence chromatin accessibility (Figure 2). For example, the bromo domain recognizes an acetylated lysine residue [12]. It occurs in chromatin-modulating proteins, such as general control of amino-acid synthesis 5-like 1 (GCN5), some of which also have intrinsic HAT activity [13]. A search of the SMART genome database [14,15] indicates that there are ≤72 bromo-domain-containing proteins in the human genome. By contrast, methylated lysine residues are recognized by chromodomain proteins, which include the family of heterochromatin protein 1 (HP-1) proteins [16], and there are ~45 entries on the SMART database for potential chromo-domain-containing proteins. These assumptions on the number of bromo-domain and chromodomain proteins imply that there is a high degree of complexity in the proteins that recognize these modifications.

Whereas the molecular and functional characterization of chromatin-regulating enzymes and cofactors is of intrinsic biological importance, it is increasingly clear that this knowledge offers great potential from medical and therapeutic standpoints, particularly in cancer. This is because in normal cells chromatin is regulated tightly in response to several physiological processes, including cell-cycle progression, the checkpoint response to DNA damage, DNA replication and chromosome stability [17,18]. Many of these fundamental processes are subject to aberrant control in cancer, partly because of mutations in genes that encode key regulatory proteins. Thus, an increasing number of examples have been identified in which mutations in cancerous cells affect chromatin regulation, sometimes directly. For these reasons, there is currently great interest in exploiting chromatin as a therapeutic target.

Here, we provide an overview of chromatin control, focusing on the most important mechanisms that impart regulation and dictate gene activity. We outline aberrations that occur in chromatin-regulating proteins in cancer, and comment on progress in exploiting this information to develop new types of cancer medicines.

HATs

It has been known for some time that the acetylation level of histones correlates with transcriptional activity [19,20]. The ε-amino group of specific lysine residues in the tail region of the four core histones becomes acetylated. Originally, this was believed to loosen chromatin by neutralizing the positive charge of lysine and, thereby, facilitating transcription [21]. However, regulation of the higher-order folding of chromatin, together with subsequent influence on compaction and condensation, is likely to have a greater role in mediating the effects of acetylation [22]. Superimposed on the global role of acetylation, it appears that histone-specific acetylation, particularly of H2A and H2B, has a significant role in destabilizing the DNA–nucleosome interaction.

An expanding group of transcriptional regulators, mostly co-activators, possess intrinsic HAT activity. One of the first groups to be identified, the p300/cAMP response element binding protein (CREB)-binding protein (CBP) family, acetylates histones and several other substrates [23]. Consistent with these observations, p300/CBP proteins are involved in diverse physiological processes, such as proliferation, differentiation and apoptosis [24]. A variety of other co-activator and related proteins also possess HAT activity, including TAF250, p300/CBP-associated factor (PCAF), hGCN5 and

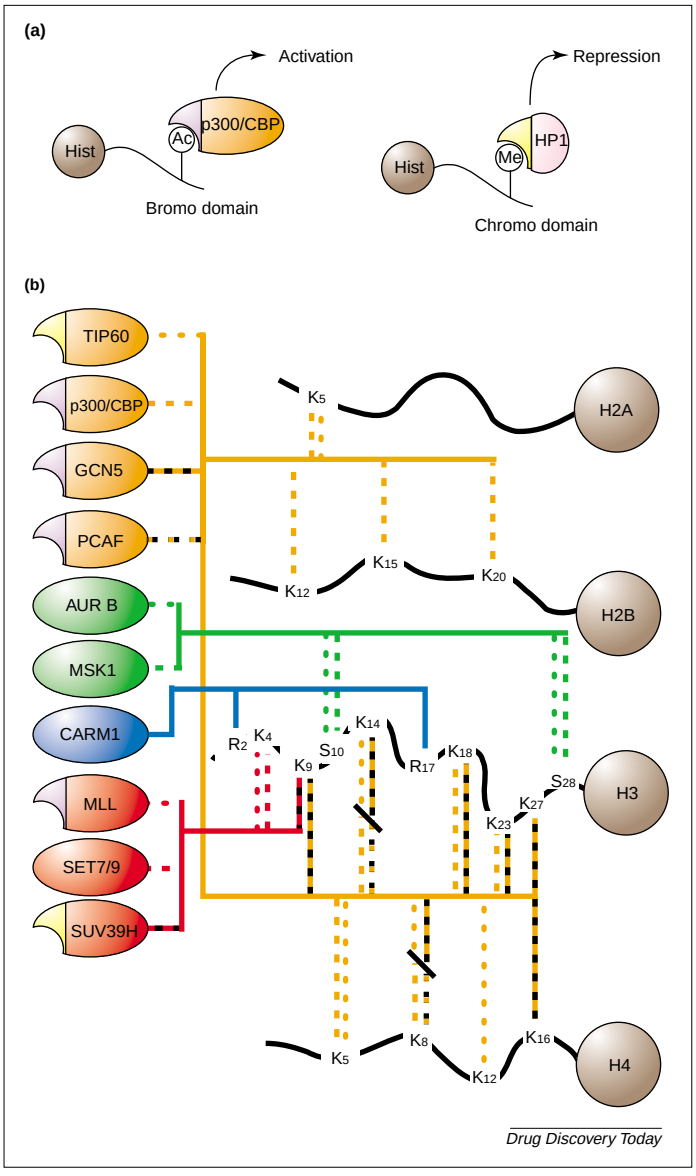

FIGURE 2

Posttranslational modifications of histone tails. (a) The bromo domain (purple) in, for example, p300/CBP recognizes acetylated lysine, whereas the chromo domain (yellow) in, for example, HP1 binds methylated lysines. Acetylation usually results in transcriptional activation and recognition of methylated lysine residues by HP1 (heterochromatin protein 1) transcriptional silencing. **(b)** Acetylases (orange), kinases (green), arginine methylases (blue) and lysine methylases (red) together with the residues that are modify in histone tails. Representative examples of the key enzyme families involved in the modifications are indicated, together with the presence of either a bromo (purple) or chromo (yellow) domain. The target residues on histone tails for the enzymes are indicated by the patterned lines next to the enzyme. For example, TIP60 targets histones: H2A Lys 5; H3 Lys 14; and H4 Lys 5,8 and 12 (indicated by the dotted orange line). H3 Lys 14 and H4 Lys 8 are acetylated by TIP60 (dotted orange line), p300 (dashed orange line), GCN5 (orange and black line) and PCAF (orange and black dashed line). Similarly AUR B (aurora kinase B) and MSK1 (mitogen and stress-responsive kinase 1), the dotted green line and the dashed green line, respectively, both target H3 Ser 10 and H3 Ser 28.

60 kDa tat-interacting protein (TIP60), and many contain a bromo domain (Table 1 and Figure 2).

From the disease perspective, some HAT genes are misregulated in cancer. p300 was identified initially as a protein that binds to

TABLE 1

Acetyltransferases with a potential role in cancer

Acetyltransferase	Family	Target	Involvement in cancer
GCN5	GCN5/PCAF	H2B, H4, cMyc	Critical regulator of cell cycle and cMyc
PCAF	GCN5/PCAF	H3, H4, cMyc, p53, MyoD, E2F	Critical regulator of cell cycle, p53, E2F and cMyc
CBP	CBP/p300	H2A, H2B, H3, H4, pRb, E2F, p53, c-Myb, MyoD, AR, FoxO	Translocation: MOZ–, MORF– and MLL–p300/CBP fusons Mutation: biallelic mutation p300 epithelial cancer Inactivation: haematological malignancy
P300	CBP/p300	H2A, H2B, H3, H4, pRb, E2F, p53, c-Myb, MyoD, AR, FoxO	Translocation: MOZMORFMLL-p300/CBP fusions Mutation: biallelic mutation p300 epithelial cancer Inactivation: haematological malignancy
TIP60	MYST	H2A, H3, H4, cMyc, AR	Association with androgen receptor in prostate cancer
MOZ	MYST	H3, H4	Fusions with p300/CBP and TIF2
MORF	MYST	H3, H4	Fusions with p300/CBP
ACTR	SRC	H3, H4	Upregulation in breast cancer correlates with resistance to tamoxfen

the adenovirus E1A oncoprotein through a domain required for viral transformation, thus, implicating p300 in the oncogenic process [25,26]. CBP, the close homologue of p300, binds to protein kinase A phosphorylated CREB [27] and the chromosomal region that contains the CBP gene is translocated in haematological malignancy (Figure 3). In acute myeloid leukaemia, *CBP* is fused to *MOZ*, which encodes monocytic leukaemia zinc finger protein (MOZ) and results in a MOZ–CBP fusion protein [28].

MOZ and the related protein MOZ-related factor (MORF) are HAT proteins that contain a MYST acetyltransferase domain [29]. Other members of this family include TIP60 and HBO1 [30]. The N-terminal domains of MOZ and MORF undergo chromosomal translocation with portions of the C-terminal of the genes that encode either p300 or CBP (Figure 3), with the resulting fusion protein retaining both the MYST and HAT domains [29]. MOZ can also rearrange with transcriptional intermediary factor 2 (TIF2), a co-activator for nuclear receptors. This fusion protein recruits p300/CBP via an interaction with TIF2-forming aberrant protein complexes that are likely to have leukaemic potential [31].

It remains unclear whether the oncogenic potential of such fusion proteins results from a loss-of-function or gain-of-function. One piece of evidence that indicates a gain-of-function comes from studies on the mixed lineage leukaemia (MLL) protein, which contains a SET domain (see later). The gene that encodes MLL is translocated in leukaemia, resulting in the formation of either the MLL–p300 or the MLL–CBP fusion (Figure 3). In normal cells, MLL positively regulates transcription, involving an interaction with CBP [32]. However, overexpression of the MLL–CBP fusion protein in a mouse model induces leukaemia [33]. The presence of the MLL–CBP fusion protein in many human leukaemias might therefore contribute to leukaemiogenesis [29,34,35].

The genes that encode p300/CBP are altered in several other human tumours, including breast and colorectal tumours and glioblastomas [36]. For example, missense mutations in p300 and loss of heterozygosity around the gene that encodes p300 have been identified in solid tumours. This could reflect evidence that p300/CBP proteins possess tumour-suppressor activity, which might be related to their role in regulating both chromatin- and non-chromatin-associated proteins such as p53 [37]. Mutation in *CBP* is the genetic basis of Rubinstein-Taybi syndrome, an autosomal-dominant disease that is characterized by a complex pathology [38,39] that includes a high incidence of neoplasia [40]. Similarly, other HATs are under abnormal control in cancer, including PCAF and ACTR [41–43]. Because the human genome contains many HAT genes, it is probable that further aberrations will be described in cancer.

HDACs

The acetylase activity of HATs is counter-balanced by the opposing deacetylase activity, which also plays a central role in controlling gene expression. In mammalian cells the HDAC family is divided into three classes (Table 2). Classes I and II are structurally similar, particularly across the active site and their enzyme activity is zinc-dependent [44]. By contrast, class III enzymes (known as the SIRT family because of their similarity with the yeast sirtuins) are zinc-independent and require NAD as a cofactor. To date, 11 members of classes I and II have been characterized along with seven members of class III [44–46]. HDAC11, which has been identified recently, is related to class I but might form a separate class [44,47].

Like HATs, HDACs usually occur as components of complexes of different co-repressor proteins, and are recruited to chromatin by interactions with either transcription factors or chromatin-associated complexes (Table 2 and Figure 4). The co-repressor's nuclear co-repressor (NCoR) and silencing mediator for retinoid and thyroid receptors (SMRT) recruit HDAC1 and HDAC2 through mSin3. HDAC1 and HDAC2, in turn, interact with, and maintain, the repressive activity of diverse transcription factors, such as the nuclear receptor family [48–50]. HDACs interact with a wide variety of transcription factors, so it is somewhat surprising that expression profiling by microarray analysis indicates that relatively few genes (2–10%) are regulated by inhibition of HDAC activity [51].

Generally, HDACs are overexpressed and/or misregulated in tumour cells, but there are no reports of mutations in HDAC genes. HDAC2 is implicated in the Wnt signalling pathway and is overexpressed in tumours derived from patients with familial adenomatosis polyposis (FAP) in whom the adenomatosis polyposis coli (APC) tumour-suppressor gene is mutated [52]. In leukaemia, the interaction of HDAC with the retinoic acid receptor (RAR) is subject to aberrant control [53]. A heterodimeric complex between the RAR and the retinoid X receptor (RXR) is required for

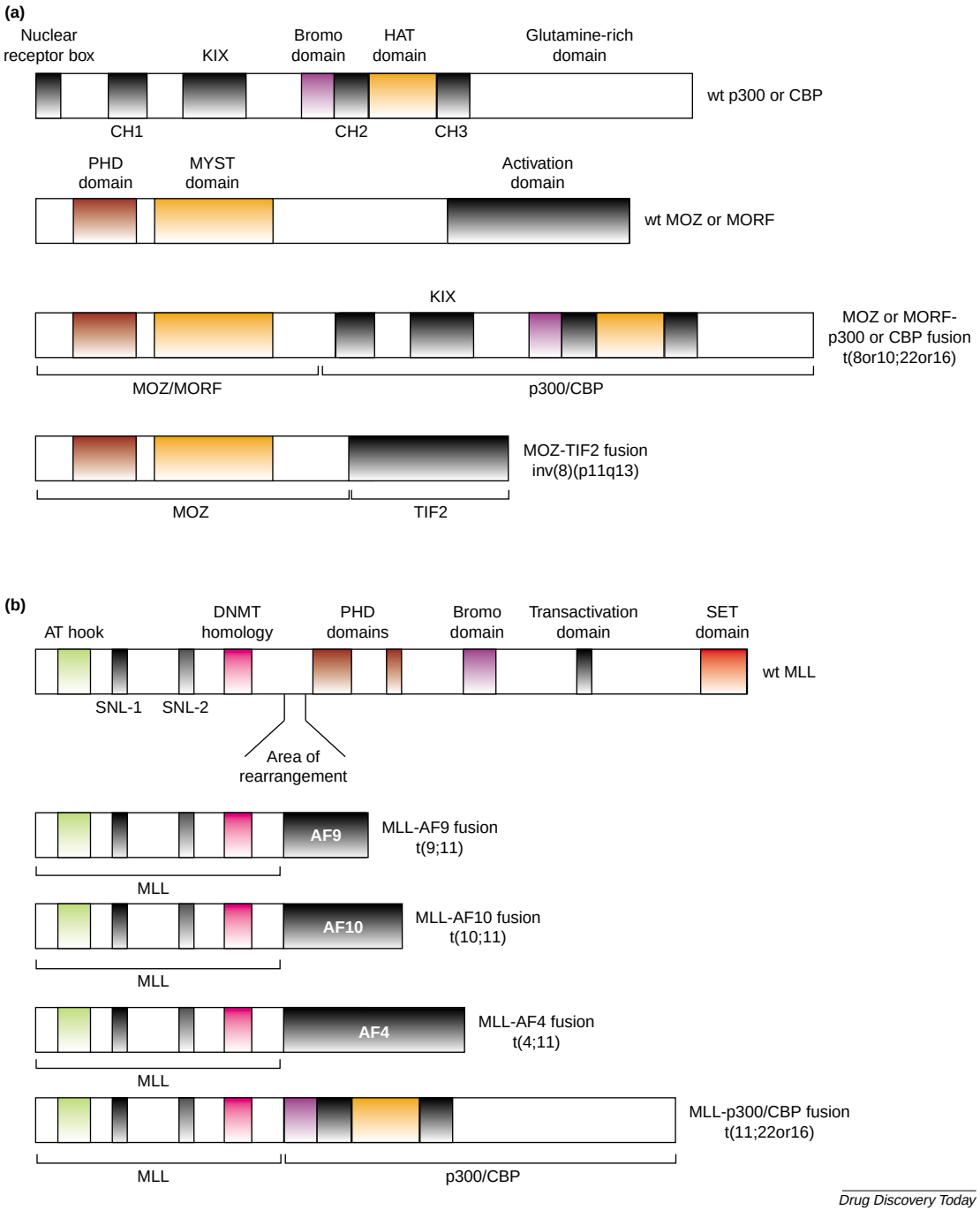

FIGURE 3

Fusion proteins that result from chromosomal translocations of genes that encode chromatin-modulating proteins. (a) Rearrangements of the genes that encode MOZ and its close homologue MORF with p300/CBP (both p300 and CBP can rearrange with either MOZ or MORF) and the p300/CBP binding partner TIF2 in acute myeloid leukemia (AML) [29,36]. The resulting protein domains in both the wild-type and fusion proteins are HAT and the related MYST acetyltransferase domain (orange) and bromo domain (purple). **(b)** Rearrangements of the MLL gene in acute lymphocytic leukaemia (ALL) and AML [29,77]. The AT hook domain (green), DNA methyltransferase (DNMT) homology domain (pink), bromo domain (purple) and lysine methyltransferase (SET) domain (red) are indicated. The MLL gene can be translocated to different co-activator-encoding genes, including AF9, AF10, AF4 and p300/CBP, as indicated [77].

differentiation in response to retinoic acid (RA). In the absence of RA, the RAR–RXR heterodimer recruits a complex that consists of HDAC–Sin3–NCoR, which represses target genes that contain a RA-response element. After binding RA, the HDAC–Sin3–NCoR repressor complex dissociates and is exchanged for an activating complex composed of p300/CBP and TIF2 (Figure 4).

In leukaemia, many mechanisms that contrive to misregulate the RAR–RXR complex result in overall insensitivity of leukaemic cells to physiological concentrations of RA, maintaining them in a continual state of proliferation (Figure 4). These mechanisms include mutation of RAR subdomains, and chromosomal translocation and fusion of either the promyelocytic leukaemia (PML) or

TABLE 2

Characteristics of HDACs

HDAC	Class	Interactions	Cellular localization
HDAC 1	I	DNMT1, ATM, BRCA1, MECP2, MYOD, p53, pRb, NF-κB	Nuclear
HDAC 2	I	DNMT1, BRCA1, pRb, NF-κB, GATA2	Nuclear
HDAC 3	I	pRb, NF-κB	Nuclear and cytoplasmic
HDAC 8	I	a-SMA	Nuclear and cytoplasmic
HDAC 4	II	14–3-3, MEF2, calmodulin	Nuclear and cytoplasmic
HDAC 5	II	14–3-3, MEF2, calmodulin	Nuclear and cytoplasmic
HDAC 6	II	Ubiquitin, tubulin, PP1, dynactin, HDAC11	Nuclear and cytoplasmic
HDAC 7	II	14–3-3, MEF2, calmodulin	Nuclear and cytoplasmic
HDAC 9	II	14–3-3, MEF2, calmodulin	Nuclear and cytoplasmic
HDAC 10	II	PP1, LcoR	Nuclear and cytoplasmic
HDAC 11	I	HDAC6	Nuclear
SIRT1	III	FOXO, p53, p300	Nuclear
SIRT2	III	tubulin	Cytoplasmic
SIRT3	III	NA	Mitochondrial
SIRT4	III	NA	NA
SIRT5	III	NA	NA
SIRT6	III	NA	NA
SIRT7	III	NA	NA

Abbreviation: NA, not available.

the promyelocytic zinc finger (PLZF) gene to the gene encoding RAR [54]. Although the transcriptional role of PML is not understood clearly, it binds to HDAC and the RAR–PML fusion blocks the response to RA in leukaemic cells. This effect is likely to occur through the stable recruitment of HDAC–Sin3–NCoR repressor complex by the RAR–PML fusion, which dissociates from the RAR–RXR complex at high, pharmacological concentrations of RA. Consistent with these observations, patients treated with pharmacological doses of RA often progress into remission [55]. Translocation between RAR and PLZF might block differentiation through a similar mechanism but this fusion protein appears to be less sensitive to RA [56]. The role of HDAC in signalling by RA and its misregulation in leukaemia has led to preclinical studies to assess the combination of HDAC inhibitors and RA.

It is noteworthy that, in addition to a role in facilitating proliferation, HDACs are also implicated in tumour-suppressor activity. HDAC1 associates with a complex that is formed between the retinoblastoma tumour suppressor protein (pRb) and the E2F transcription factor, which represses E2F target genes and causes cell-cycle arrest [57,58].

HDAC enzymes have complex levels of control. Whereas members of the class I group are nuclear, class II enzymes are controlled by nucleo–cytoplasmic transport. In proliferating myoblasts, HDAC4, HDAC5 and HDAC7 localize to the nucleus where they interact with HDAC3 and inhibit the myogenic transcription factor MEF2. Upon differentiation to myotubes, HDACs dissociate from MEF2 and are sequestered in the cytoplasm by interacting with 14-3-3 proteins, allowing MEF2 to recruit p300 and activate transcription [45,59].

The ability of HDACs to repress transcription is linked with another epigenetic modification that is mediated through DNA methylation [60]. Although most of the human genome is depleted of CpG dinucleotides, small regions of DNA, known as CpG islands contain stretches of CpGs that remain free from methylation [61,62]. Frequently, CpG dinucleotides are located close to the promoters of genes and methylation of the cytosine base is associated with gene silencing [63,64]. This occurs, in part, because methylated CpGs are recognized by a group of DNA-binding proteins, such as methyl CpG-binding protein 2 (MeCP2), that recruit HDAC co-repressor complexes, thus, favouring transcriptional repression [65,66]. There is, therefore, a tight interplay between DNA methylation and HDAC activity in gene silencing, which, in turn, alerts us to the possibility of combining drugs that target different levels of epigenetic control for maximum clinical benefit.

Lysine methyltransferases

Histone tails can be mono-, di- and tri-methylated on the ε-amino group of lysine residues, and either mono- or di-methylated on arginine residues [67]. Most lysine methyltransferases are characterized by a conserved SET domain (Figure 2) and, from the cancer perspective, SET domain proteins are under abnormal control in tumours (Table 3) [68]. Depending on the context, lysine methylation provides either an activating or repressing modification [69]. Thus, tri-methylation of Lys9 in histone H3 by SUV39H1 (suppressor of variegation 3–9 homologue 1), the mammalian homologue of the *Drosophila* position effect variegation modifier protein Su(var) 3–9, is associated primarily with transcriptional silencing, whereas Lys4 methylation by Set9 correlates with transcriptional activation [70].

Proteins of the polycomb group function in transcriptional repression and occur in protein complexes that, in humans, include the SET-domain protein – enhancer of zeste homologue 2 (EZH2) [71]. EZH2 tri-methylates Lys27 in histone H3 and is overexpressed in several cancers [72–74]. In prostate cancer, microarray analysis of tumour samples has identified expression of EZH2 as a marker for poor prognosis, and reducing the concentration of EZH2 prevents

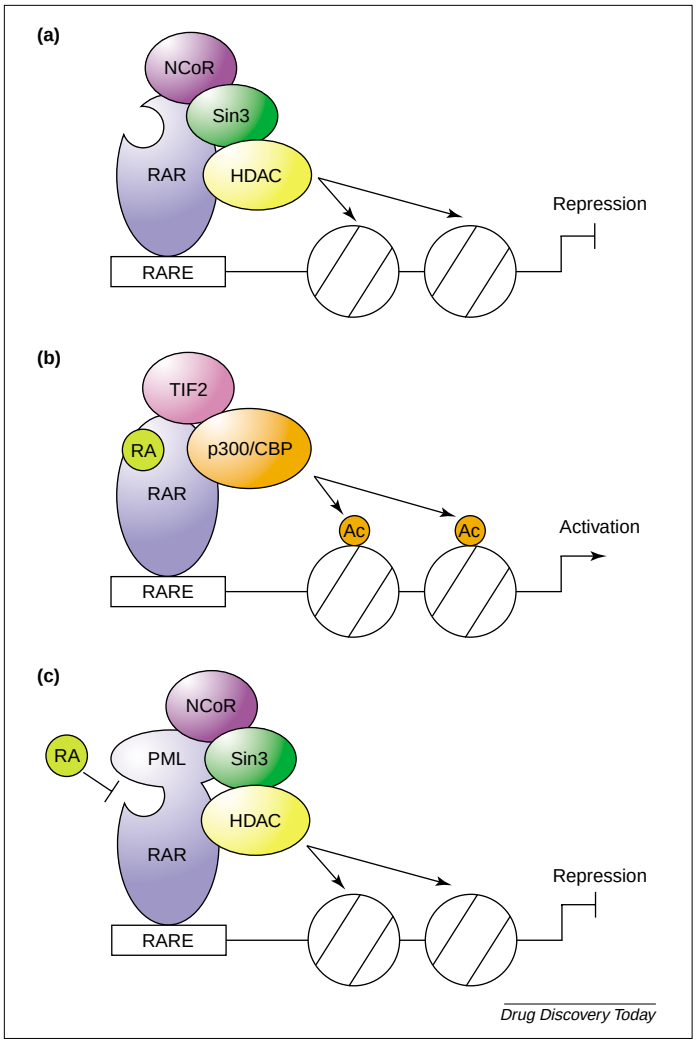

FIGURE 4

Role of HDAC in RA signalling in leukaemia. (a) An RAR–RXR heterodimer recruits a complex that consists of HDAC, Sin3 and NCoR, which represses target genes regulated by a retinoic-acid-responsive element (RARE). **(b)** Upon binding RA, a co-activator complex is recruited, which leads to transcriptional activation and acetylation of chromatin. **(c)** Chromosomal translocation results in the RAR–PML fusion protein, which stably recruits the HDAC–Sin3–NCoR repressor complex and so causes insensitivity to RA. Only at higher pharmacological concentrations of RA does the RAR become transcriptionally active.

proliferation of cancer cells [75]. EZH2 might, therefore, silence genes that delay cell growth.

In contrast to the polycomb-group proteins, the trithorax group is involved principally in activating transcription [76]. The gene that encodes MLL is a human homologue of *Drosophila* trithorax and, as mentioned above, is mutated, usually through translocation, in leukaemia. MLL contains a characteristic SET domain and methylates Lys4 in H3, leading to transcriptional activation [77].

SUV39H1 was one of the first SET proteins to be identified [78]. SUV39H1-mediated tri-methylation of Lys9 in H3 is recognized by heterochromatin protein 1 (HP1) through its chromo domain, leading to transcriptional silencing [79]. SUV39H1 interacts with pRb and acts in concert with pRb and HDAC to maintain inactivity of E2F, the cell-cycle-regulating transcription factor and, thereby, delays cell-cycle progression [80]. Consistent with this, mice that are deficient in SUV39H1 have an increased incidence of tumours [81]. Moreover, it has been suggested that maintenance of histone methylation by SUV39H1 has a role in senescence [82]. Oncogene-induced senescence (OIS) appears to have a role in constraining the progression of tumours and SUV39H1 is required for Ras-induced OIS in lymphocytes. In the absence of SUV39H1, mice succumb to invasive T cell lymphomas, indicating that methylation of Lys9 of H3 is active in a cellular-senescence programme and that SUV39H1 might provide a novel tumour-suppressor mechanism [82].

Another SET domain protein, pRb-interacting zinc finger (RIZ1), methylates Lys9 of H3 and exhibits tumour-suppressor activity [83]. RIZ1 associates with pRb and, in cancer, mutations in the gene that encodes RIZ1 affect its methyltransferase ability [84]. Introducing RIZ1 into proliferating cells causes cell-cycle arrest and apoptosis [85], and RIZ1-knockout mice have an increased incidence of tumours [86]. Thus, RIZ1 appears to be involved in negatively regulating proliferation.

More recently, SMYD3 has been identified as a SET domain protein that is overexpressed in hepatocellular and colorectal carcinoma [87], deregulation of the pRb/E2F pathway is associated with increased expression of the gene encoding SMYD3 [88]. SMYD3 associates with RNA polymerase II via the RNA helicase HELZ and methylates Lys3 in H4. A separate domain in SMYD3, the MYND domain, allows SMYD3 to be targeted to promoters. Furthermore, SMYD3 has oncogenic activity in a colony-forming assay, and siRNA against SMYD3 delays cell-cycle progression [87].

The action of lysine methyltransferase is counter-balanced by demethylases such as lysine-specific demethylase (LSD1) [89–91]. LSD1 has attracted considerable interest because inhibition of demethylase activity might retain chromatin methylation and repression of genes involved with cancer, such as the androgen receptor [92]. LSD1 co-localizes with androgen receptors in prostate cancer and stimulates receptor-dependent transcription.

Overall, several SET domain proteins are deregulated in cancer, frequently through gene-mutational events [68]. The effect of mutation is gene specific: it can either promote oncogenesis or inactivate tumour-suppressor activity.

TABLE 3

Methyltransferases with a potential role in cancer

Methyltransferase	Family	Target	Involvement in cancer
MLL	SET1	(DNA) H3, K4	Negatively regulates Hox genes. SET domain lost in translocation fusion proteins
SMYD3	NA	H3, K4	Upregulates oncogenes and cell cycle genes
hDOT1L	non-set (HMT)	H3, K79	Interacts with AF10 (comon fusion of MLL)
Suv39h1	SUV39	H3, K9	Non-Hodgkin lymphoma, involved with pRb/E2F

Abbreviation: NA, not available.

Arginine methyltransferases

Protein arginine methyltransferases (PRMTs) catalyze the transfer of methyl groups to the guanidino nitrogen of arginine residues [93]. PRMTs are divided into two groups depending on whether they catalyze asymmetric or symmetric di-methylations. To date, the region required for catalytic activity and binding of *S*-adenosyl-L-methionine cofactor is similar in all PRMTs [94,95], but only two, PRMT5 and PRMT7, catalyze symmetric dimethylation [94].

Currently, no mutations have been identified in PRMTs in tumour cells but, in addition to their role in histone modification, they target several proteins that influence proliferation. For example, PRMT1 regulates the nucleo–cytoplasmic transport of RNA-processing proteins [96] and functions in interferon-mediated signal transduction, in part, through the methylation of the STAT1 transcription factor [97,98]. Methylation of STAT1 increases DNA binding and transcriptional activity, and inhibition of PRMT1 blocks the interferon response. In addition, PRMT1 methylates Arg3 in H4, facilitating acetylation of H4 by p300 and leading to transcriptional activation [99]. This might account for the ability of PRMT1 to co-activate nuclear hormone receptor activity when co-expressed with p160 family of transcriptional co-activators [100].

Another PRMT, PRMT4 [also known as coactivator-associated arginine methyltransferase 1 (CARM1)] also interacts with the p160 family of nuclear receptor co-activators, again enhancing transcription [101]. PRMT4 methylates Arg17 in H3, prompting activation [102,103], and non-histone proteins, including the p300 HAT, are also methylated by PRMT4 [104,105]. Consistent with these observations, PRMT4 cooperates with p300 in the activation of nuclear receptor transcription [100].

PRMT5 methylates H2A and H4 *in vitro* [106–108] and is involved with downregulation of cyclin E transcription through a mechanism that probably involves E2F [109]. In addition, PRMT5 co-purifies with components of the SWI/SNF complex where it methylates histone H3 and H4 [110]. It is possible that PRMT5 negatively regulates proliferation by inactivating cyclin E expression and by interfering with Ras-mediated oncogenesis [111].

Whereas studies of PRMTs are in their infancy, it is likely that they take on diverse roles in integrating chromatin with gene expression and other cellular processes. As such, PRMTs are likely to provide valuable targets in the design of new cancer therapies.

Phosphokinases

Phosphorylation of chromatin has an important role in mitosis and transcriptional control. The phosphorylation of Ser10 in H3 is associated with chromosome condensation during mitosis, and in interphase cells facilitate transcriptional activation in response to a host of different signalling events [112]. At least two groups of kinases are likely to be involved in regulating the phosphorylation of Ser10. These include aurora kinases, which are required for chromosome architecture and appropriate mitosis and meiosis in eukaryotic cells, and mitogen and stress responsive kinases, which target Ser10 during transcriptional activation [113].

Although the three members of the aurora kinase family in mammalian cells, Aurora A, Aurora B and Aurora C, share a similar organization, their subcellular distribution and expression patterns are distinct. Aurora A is involved principally with centrosome maturation and spindle assembly. Aurora B, which has been linked with the phosphorylation of H3, is involved with chromosome condensation and segregation and with the spindle checkpoint [114]. Aurora C has a restricted expression pattern and its functional role remains to be characterized [114].

Aurora kinases are implicated in carcinogenesis because they are overexpressed frequently in tumour cells [115,116]. Increased levels of Aurora A can override the spindle checkpoint and confer resistance to the chemotherapeutic agent paclitaxel [117]. In colorectal cancer and oesophageal squamous cell carcinoma an Aurora A allele in which there is an Ile31Phe polymorphism, which is less susceptible to degradation, is amplified selectively [118]. Because of their abnormal regulation in cancer, aurora kinases are attracting attention as potential anticancer targets.

The phosphorylation on Ser10 in H3, that affects transcriptional activation, involves the mitogen and stress-responsive kinases MSK1 and MSK2 [119]. Ser10 is close to several other modified residues, such as Lys9, which can be acetylated or methylated (Figure 1 and Figure 2). Evidence from several reports supports the contention that this close proximity might enable different modifications within the histone tail to influence each other (frequently referred to as crosstalk). For example, methylation of Lys9 is influenced strongly by phosphorylation of Ser10, because Ser10 phosphorylation impedes methylation of Lys9 and promotes the acetylation of Lys14, which correlates with transcriptional activation. Thus, yeast enzymes that phosphorylate Ser10 and acetylate Lys14 (Snf1 and GCN5, respectively) cooperate in transcriptional activation [120].

Studies such as these highlight the different layers of crosstalk between the posttranslational modifications of histones and the interplay between upstream signalling pathways. At a therapeutic level, it is possible that a combination of drugs that target different steps in the modification and regulation of chromatin will have synergistic effects in impeding tumour-cell growth, an idea which is gathering increased momentum.

Cancer-drug discovery

Drugs that regulate DNA methylation validate epigenetic control as an important mechanism in cancer and a viable therapeutic target [121,122]. It has been known for some time that DNA methylation is abnormal in tumour cells [123] where hypermethylation of CpG islands prompts transcriptional silencing [62]. At least three methyltransferases, DNMT1, DNMT2 and DNMT3, lay down the pattern of genomic methylation [124] and, in cancer cells, there are many examples of tumour-suppressor genes that are silenced by this mechanism, including *Rb*, *APC* and *BRCA1* [125–127]. In colorectal cancer the methylation of *MLH1* contributes to defective mismatch repair and microsatellite instability [128].

Drugs that inhibit DNA methyltransferase activity, such as 5-azacytidine and decitabine, reactivate methylation-dependent silenced genes in tumours and some of these agents have been evaluated in clinical trials [122]. Reports vary on their clinical activity, both as stand-alone agents and combination therapies [60,129–133]. Toxicity is a long-standing concern and, therefore, recent advances have focused on less toxic compounds with demethylating activity [134,135]. Nevertheless, the US Food and Drug Administration recently approved 5-azacytidine for the treatment of myelodysplastic syndrome, validating epigenetic control as a cancer target (www.fda.gov) [121].

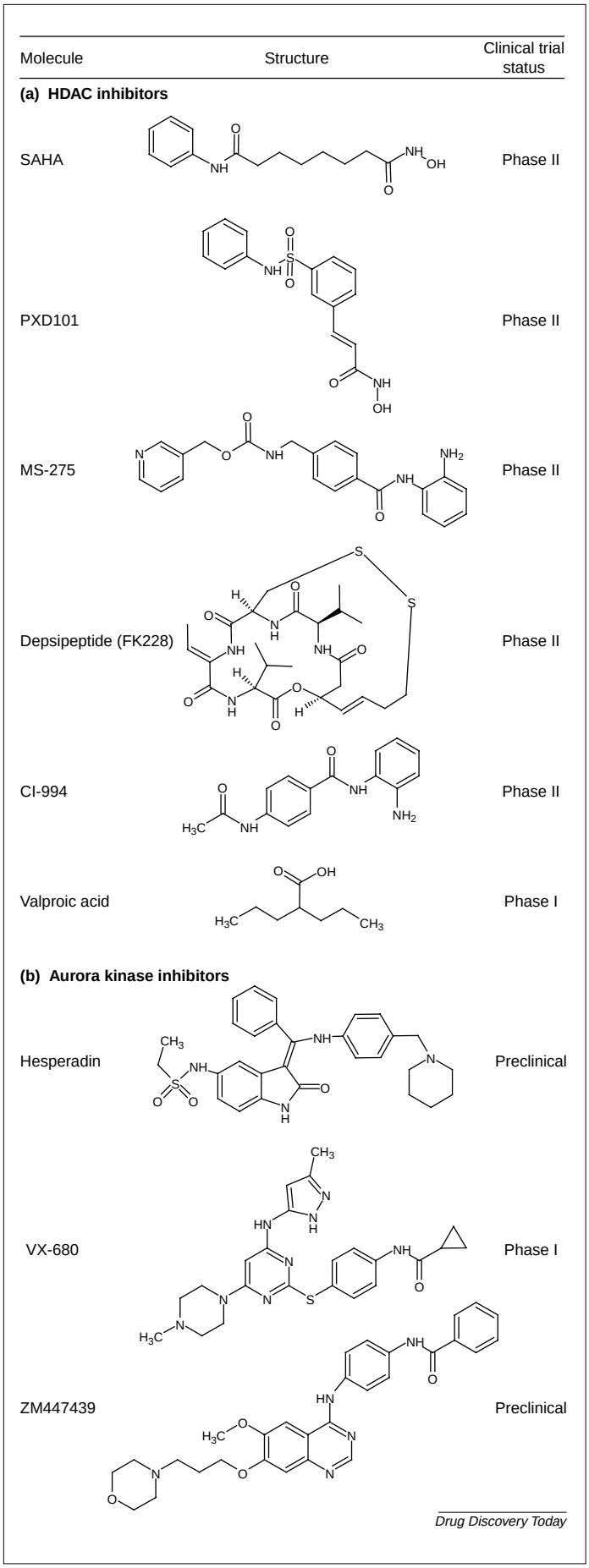

FIGURE 5

Compounds in either pre-clinical or clinical studies that target HDAC and aurora kinase in cancer. (a) Structures of some of the HDAC inhibitors and their stages of clinical development. **(b)** Summary of aurora kinase inhibitors and their stages of development, together with specificity of compound.

In addition to inhibitors of DNA methylation, remarkable progress has been achieved in developing drugs that target HDACs and HDAC inhibitors are beginning to validate chromatin control as an important cancer target. HDACs have been implicated widely in growth and transcriptional control, and inhibition of HDAC activity causes apoptosis in diverse types of tumour cells [136]. The naturally occurring fungal antibiotic trichstatin A (TSA) was one of the first inhibitors of HDAC to be identified as an antiproliferative agent and it has proved to be an invaluable tool to study HDACs and their role in cancer-cell proliferation. TSA docks into the active site of the enzyme, chelating the essential Zn^{2+} ion and inhibits enzyme activity with an IC_{50} in the low nM range. Of the hydroxamic acid-based HDAC inhibitors in clinical development (Figure 5) the most advanced [suberoylanilide hydroxamic acid (SAHA) and PXD101] have both reached Phase II trials. SAHA has demonstrated a well-tolerated safety profile and encouraging results in cutaneous T cell lymphoma [137–139]. Similarly, PXD101 has low toxicity and is being evaluated in an extensive series of clinical studies.

An early concern relating to hydroxamic-based HDAC inhibitors reflected their pharmaco-kinetic properties because the compounds have a relatively short half-life. However, it is encouraging that the pharmaco-dynamic effects of HDAC inhibitors in clinical studies are relatively long-lived, which perhaps reflects a mechanism in which a short exposure to an HDAC inhibitor causes a change in acetylation that is maintained over a longer period because of the interplay with other chromatin modifications. Under these circumstances, the interplay and crosstalk between histone modifications might lock the increased acetylation level in place and, thus, retain altered levels of gene expression for longer time periods.

Less potent than the hydroxymic acid-based compounds are the aliphatic acid derivatives, such as valproic acid (VPA) and butyric acid. Although currently in clinical trials for cancer because it inhibits HDAC [140,141], VPA has also been widely used as a treatment for epilepsy [142]. Pharmacodynamic measurements in the clinical setting support the hypothesis that HDAC is a target of VPA [143]. Other HDAC inhibitors have been described, including the depsipeptide FK-228, currently in Phase II trials (Figure 5).

HDAC inhibitors block cell-cycle progression and induce apoptosis. However, it is unclear whether acetylation of chromatin and the subsequent influence on gene activity is the primary mechanism of drug action, or whether there is a more limited repertoire of targets. In support of this idea, microarray analysis indicates that inhibition of HDAC causes a limited change in gene expression [51]. Equally important, therefore, might be the non-histone targets that are influenced by acetylation, including master cell-cycle regulators such as E2F and tumour suppressors such as p53 and pRb [144–146]. For these proteins, acetylation augments tumour-suppressor activity, which provides a plausible mechanism to account for the effects of HDAC inhibitors on proliferation.

Considering their clinical utility, it is encouraging that HDAC inhibitors are active in cell lines that are resistant to existing

chemotherapies and resistance to HDAC inhibitors has not, so far, been documented in either laboratory or clinical settings. In fact, HDAC inhibition has been exploited to alter the resistance of tumour cells to other classes of drugs. For example, Gleevec-resistant Bcr/Abl human CML (chronic myeloid leukaemia) cells are resensitized to Gleevec by co-treatment with SAHA [147], and TSA enhances the expression of the oestrogen receptor in breast cancer cells, with subsequent resensitization to tamoxifen [148].

Frequently, cancer drugs are used in combination, partly to reduce toxicity and also to maximize the clinical benefit and tumour response during disease progression. From a scientific standpoint, and considering the interplay between the different levels of control of chromatin and gene expression, it should be possible to predict appropriate epigenetic mechanisms to target when combining therapies for maximum clinical effect. Based on our knowledge of the interplay between methylation of DNA and transcriptional silencing, synergistic effects of combining a DNA methyltransferase inhibitor with an HDAC inhibitor [149] make good sense. Similarly, the increased efficacy observed in leukaemia when RA is combined with an HDAC inhibitor can be explained from our understanding of the role of HDAC in regulating the activity of RAR [53]. We await results from other combination studies that target discrete epigenetic processes and anticipate that such information will highlight either improved efficacy or expanded clinical utility that can be harnessed subsequently in therapeutic strategies.

Although there are no reports on the effect of inhibitors of lysine histone methyltransferase, it is generally anticipated that, when available, these inhibitors will also exhibit antiproliferative effects. EZH2 and SMYD3 are overexpressed in several cancers and their SET domain is required for increased proliferation [73,87]. Inhibitors that target these methyltransferases might impede tumour-cell growth, perhaps by catalytically inactivating the activity of their SET domain. Several reports document inhibitors of PRMTs [150] and, as with other mechanisms of epigenetic control, we anticipate that they also will have antiproliferative effects. HAT inhibitors that exhibit specificity for p300/CBP and PCAF have been described [151–153]. A natural product, Garcinol, inhibits p300 and PCAF and induces apoptosis [154]. Whereas studies on inhibitors of lysine and arginine methyltransferases and acetyltransferases are in their infancy, it is clear from the limited information available that they are likely to provide interesting agents to pursue in the cancer clinic.

The phosphorylation of Ser10 in H3 is mediated through different kinase networks that are tied into a variety of functional outcomes. The aurora kinases are particularly relevant to cancer drug discovery and have attracted considerable attention as therapeutic targets. Several aurora kinase inhibitors are under development (Figure 5). VX680 results from a structure-based design approach using the three-dimensional structure of the catalytic domain of Aurora A. VX680, which blocks cell-cycle progression, induces apoptosis in range of cancer cell lines and delays tumour progression in xenograft assays, has recently entered Phase I clinical trials. Two other inhibitors, Hesperadin and ZM447439, are selective for Aurora B and, similarly, induce cell-cycle arrest [155,156]. It has been suggested that aurora kinase inhibitors cause aberrant mitosis and that cell-cycle arrest is mediated principally through the activation of postmitotic checkpoints that might involve p53 [114]. Although it is not clear how the effects of aurora kinase inhibition are mediated, the results obtained highlight aurora kinases and, thus, protein kinases that target histones as an attractive cancer-treatment target.

Concluding comments and future perspectives

We are embarking on a new era of cancer-drug discovery in which chromatin-regulating drugs are taking centre stage. Concerted efforts to develop HDAC inhibitors, together with the promising results from clinical trials, places HDAC inhibitors as strong contenders for the first class of chromatin drug to achieve clinical utility. Given the current interest in the other types of enzymes involved with chromatin control, it seems likely that HATs and MTases will also be tractable therapeutically and yield promising anticancer agents.

However, as we look to the future there are many outstanding questions. Using HDAC as an example (the question is equally relevant to other chromatin targets) we have to consider the ideal specificity of HDAC inhibitors. For example, is subunit specificity an advantage or will pan-inhibitors be more successful because of the reduced likelihood of selecting resistant tumour cells? The advent of inhibitors that act selectively on HDAC subunits [157,158] should make this information forthcoming in due course.

How do we identify the tumour spectrum and, within this context, the stage of disease progression where we might expect to achieve maximum efficacy with a chromatin drug? Again considering HDAC inhibitors, there are some immediate parameters that we expect to influence tumour-cell sensitivity. For example, the expression level and repertoire of HDAC subunits and the assembly of HDAC with co-repressors is likely to impact on the ability of a small-molecule to block enzyme activity. At a mechanistic level, and again a question that is particularly relevant to the development of HDAC inhibitors, how do HDAC inhibitors kill cells? HDAC inhibition elicits apoptosis but is this outcome a result of deregulating numerous survival targets or is chromatin the main target and tumour suppressors reactivated? In our opinion it more likely that HDACs provide a fundamental level of enzymatic control and that a blunt knockout of HDAC activity is sufficient to drive tumour cells into the apoptotic programme.

Biomarkers that inform on the likelihood of clinical response will provide valuable tools in stratifying tumours and achieving a successful clinical outcome. We imagine that the global acetylation level of either chromatin or, perhaps, individual cell regulators (such as E2F, pRb and p53) might be useful markers of the response to HDAC inhibitors. A recent study indicates that global histone modification (including acetylation and methylation) in prostate cancer correlates with the stage of disease and might provide informative prognostic signatures that reflect disease progression [159]. We could even integrate global histone modification into clinical strategies in which different chromatin-modifying drugs are combined in a fashion that reflects global chromatin changes during tumourigenesis.

There are many questions that remain to be answered before we understand the clinical value of chromatin as a drug target. Nevertheless, we are at an important watershed in the validation of chromatin drugs and we suggest that we are close to 'rubber stamping' chromatin regulation as a successful clinical regimen. We expect that the fever-pitch progress in chromatin drug discovery will yield new agents that provide improved, efficacious cancer drugs.

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