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Epigenetic pathway targets for the treatment of disease: accelerating progress in the development of pharmacological tools: IUPHAR Review 11

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This article, written by members of the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) subcommittee for epigenetic targets (chromatin-modifying enzymes and bromodomain-containing proteins), reviews our current understanding of their structure, pharmacology and functions, and their likely physiological roles in health and disease. More information on these families of targets can be found in the Concise Guide to PHARMACOLOGY (http://onlinelibrary.wiley.com/doi/10.1111/bph.12445/abstract) and for each member of the families in the corresponding database (http://www.guidetopharmacology.org/GRAC/ReceptorFamiliesForward?type=ENZYME&familyId=865; http://dev.guidetopharmacology.org/GRAC/ReceptorFamiliesForward?type=OTHER&familyId=866).

The properties of a cell are determined both genetically by the DNA sequence of its genes and epigenetically through processes that regulate the pattern, timing and magnitude of expression of its genes. While the genetic basis of disease has been a topic of intense study for decades, recent years have seen a dramatic increase in the understanding of epigenetic regulatory mechanisms and a growing appreciation that epigenetic misregulation makes a significant contribution to human disease. Several large protein families have been identified that act in different ways to control the expression of genes through epigenetic mechanisms. Many of these protein families are finally proving tractable for the development of small molecules that modulate their function and represent new target classes for drug discovery. Here, we provide an overview of some of the key epigenetic regulatory proteins and discuss progress towards the development of pharmacological tools for use in research and therapy.

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Abbreviations

α-KG, α-ketoglutarate; ADMA, asymmetrical- dimethylarginine; Apo-A1, apolipoprotein A1; BCP, bromodomain-containing protein; BET, bromodomain and extraterminal; BRD, bromodomain; DNMT, DNA-methyltransferase; EAE, experimental autoimmune encephalomyelitis; FAD, flavin adenine dinucleotide; HDAC, histone deacetylase; IPF, idiopathic pulmonary fibrosis; Jmj, jumonji C-domain; KAT, lysine acetyltransferase; KDAC, lysine deacetylase; KDM, lysine-specific demethylase; KMT, lysine methyltransferase; MMA, mono-methylated arginine; NET, neutrophil extracellular trap; NMC, NUT midline carcinoma; NOG, N-oxalylglycine; NUT, nuclear protein in testis; PAD, peptidyl-arginine deiminase; PCPA, trans-2-phenylcyclopropylamine; PRMT, arginine methyltransferase; SAHA, suberoylanilide hydroxamic acid; SAM, S-adenosyl methionine; SDMA, disymmetrical-dimethylarginine; SIRT, sirtuin; SNP, single nucleotide polymorphism; TET, ten-eleven translocation

Links to online information in the IUPHAR/BPS Guide to PHARMACOLOGY

TARGETS

ASH1L	HDA8	PRDM2
ATAD2	HDAC9	PRMT1
ATAD2B	JMJD1C	PRMT10
BAZ1A	KAT2A	PRMT2
BAZ1B	KAT2B	PRMT3
BAZ2A	KAT5	PRMT5
BAZ2B	KAT6A	PRMT6
BPTF	KAT6B	PRMT7
BRD1	KAT7	PRMT8
BRD2	KAT8	SETD1A
BRD3	KDM1A	SETD1B
BRD4	KDM1B	SETD2
BRD7	KDM2A	SETD7
BRD8	KDM2B	SETD8
BRD9	KDM3A	SETDB1
BRDT	KDM3B	SETDB2
BRPF1	KDM4A	SIRT1
BRPF3	KDM4B	SIRT2
BRWD1	KDM4C	SIRT3
BRWD3	KDM4D	SIRT4
CARM1	KDM4E	SIRT5
CECR2	KDM5A	SIRT6
CLOCK	KDM5B	SIRT7
CREBBP	KDM5C	SMARCA2
DOT1L	KDM5D	SMARCA4
EHMT1	KDM6A	SMYD2
EHMT2	KDM6B	SP100
EP300	KDM7A	SP110
EZH2	KDM8	SP140
FBXO10	KMT2A	SP140L
FBXO10	KMT2B	SUV39H1
FBXO11	KMT2C	SUV39H2
GTF3C4	KMT2D	SUV420H1
HAT1	KMT2E	SUV420H2
HDAC1	NCOA1	TAF1
HDAC10	NCOA2	TAF1L
HDAC11	NCOA3	TRIM24
HDAC2	NSD1	TRIM28
HDAC3	PBRM1	TRIM33
HDAC4	PHF2	TRIM66
HDAC5	PHF8	ZMYND11
HDAC6	PHIP	ZMYND8
HDAC7		

LIGANDS

AMI-1
anacardic acid
AZ505
benzo[d]imidazole inhibitors of PRMT4 from BMS
BIX-01294
BMS pyrazole inhibitor 7f
BRD4770
bromo-deaza-SAH
butyric acid
C21
C646
Compound 1 (allosteric)
curcumin
daminozide
EI1
entinostat
epigallocatechin-3-gallate
EPZ-5676
EPZ-6438
garcinol
GSK126
GSK-J1
H3-CoA-20
I-BET-762
Lys-CoA
mocetinostat
nahuoic acid A
NCL-1
OG-L002
PBIT
plumbagin
RM65
rocilinostat
romidepsin
RVX 208
SGC0946
trichostatin A
UNC0638
UNC0642
valproic acid
vorinostat

This table lists protein targets and ligands which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013a, Alexander *et al.*, 2013b).

Introduction

The properties of a cell are determined by its specific genetic material and the pattern in which its genes and, ultimately, proteins are expressed. While the transmissibility of the genome is well recognized, the fact that cellular phenotypes can remain stable through cell division – a dividing T-cell yields two T-cells, a dividing hepatocyte yields two hepatocytes, etc. – indicates that specific gene expression patterns are also heritable in daughter cells. The latter type of inheritance is termed epigenetic (literally, 'above genetic') because the generation and maintenance of differentiated cell phenotypes is not due to changes in the nucleotide sequence. While heritable, epigenetic memory is also malleable, in that gene expression patterns can change in response to environmental stimuli. This allows for the development of different cell lineages during processes such as embryogenesis or haematopoiesis, and also for more subtle changes in cell function in response to physiological requirements, or importantly pathological stress that occurs during adaptive immunity.

Epigenetic control of gene expression is linked to the manner in which eukaryotic DNA associates with nuclear proteins, in a structure known as chromatin. The basic unit of chromatin is the nucleosome, which consists of approximately 147 bp of DNA wrapped around an octamer of core histones (which includes two each of histones H3, H4, H2A and H2B) (Luger *et al.*, 1997; Davey *et al.*, 2002). Further higher order structuring of nucleosomes through interactions with additional histone and non-histone proteins allows for tight compaction of the DNA into the limited volume of the nucleus (Talbert and Henikoff, 2010). In addition to permitting efficient DNA packing, chromatin provides an intricate scaffold for interacting with nuclear proteins such as those of the gene transcription machinery. Importantly, chromatin is subject to modifications that generate highly ordered sophisticated heterogeneity in its structure and, as a consequence, in the potential for different gene regions to be expressed. This recognition of and response to specific combinatorial patterns of modifications allows the complex epigenetic code to be translated into selective control of coordinated clusters of genes in transcriptional modules (Taverna *et al.*, 2007; Hardison and Taylor, 2012).

Chromatin can be altered by modifications to both the DNA and the associated histone proteins. At the level of the DNA, the main modification described so far is methylation of cytosine residues, which occurs predominantly in the context of the dinucleotide sequence CpG and is mediated by members of the DNA-methyltransferase family (DNMT1, DNMT3A, DNMT3B) (Okano *et al.*, 1999; Pradhan *et al.*, 1999). DNA methylation leads to suppression of gene transcription through a number of mechanisms, including direct inhibition of transcription factor binding, which is necessary for recruitment of the transcription machinery, and attraction of methyl-CpG-binding domain-containing proteins that associate with transcriptionally repressive protein complexes (Reddington *et al.*, 2013). Methyl marks can be removed from DNA in a passive manner through a failure to remethylate daughter DNA strands during cell division, or in an active process involving ten-eleven translocation (TET1-3) family proteins or DNA glycosylases and repair-mediated excision of modified bases (He *et al.*, 2011b; Ito *et al.*, 2011; Maiti and Drohat, 2011; Spruijt *et al.*, 2013). Notably, TET enzymes generate intermediates in the pathway between 5-methylcytosine and cytosine, including 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxycytosine, which can be detected in cellular chromatin and may serve as epigenetic marks in their own right (Delatte and Fuks, 2013). The therapeutic potential of targeting DNA methylation including the activity of licensed pan-DNMT inhibitors to impact certain cancers has been reviewed recently (Carey *et al.*, 2012), and will not be discussed further here.

Histone modification has been studied most extensively for the core histones of the nucleosome. Many different amino acids in these proteins are subject to post-translational modification, although the majority of these occur in the N-terminal or C-terminal 'tails' which extend outside of the main, globular histone domains (Kouzarides, 2007). By contrast to the relatively limited complexity of DNA methylation, histones can be modified in a wide variety of ways; by some accounts up to 60 different chemical modifications of histones have been documented, including acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP ribosylation, crotonylation and biotinylation (Stanley *et al.*, 2001; Kouzarides, 2007; Tan *et al.*, 2011; Nikolov and Fischle, 2013). As discussed further below, several families of enzymes responsible for the addition (writers) or removal (erasers) of these modifications have been identified. The multiplicity of modifications and amino acid substrates can be arranged into a very high number of distinct combinations, which has prompted the hypothesis that the different patterns represent a 'histone code' that provides specific instructions for a given region of DNA (Strahl and Allis, 2000). Although there is presently little understanding of a detailed code, the functional relevance of histone modification to gene regulation is evident from observed correlations between the presence of certain combinations of marks and gene expression.

Histone modifications are thought to impact gene expression in two broad ways. First, certain modifications such as acetylation alter the net charge of the histone, weakening the DNA–histone interaction and yielding a chromatin structure that is more open and hence accessible to transcription factors and gene expression machinery (Hong *et al.*, 1993). Second, and potentially more importantly, histone modifications serve as recognition marks for proteins termed epigenetic 'readers', which can specifically bind to these modified amino acids (Taverna *et al.*, 2007). Thus, while writers and erasers produce the histone code, it is the epigenetic readers that decipher and translate this information (Figure 1). Reader proteins typically possess activities that lead to activation or suppression of gene expression, and/or are able to recruit other proteins that possess such functions. As is the case for the epigenetic writers and erasers, large families of readers able to recognize different modified amino acids have been identified and recently reviewed (Arrowsmith *et al.*, 2012).

Together, DNA and histone modifications help control the 'transcribability' of genes. Thus, linked to the state of the chromatin, a gene or gene cluster may be silenced, constitutively expressed or poised for expression (or suppression) in response to a specific cell signal (Kouzarides, 2007). The dynamic nature of chromatin modifications provides a mechanism for cells to adapt their gene expression pattern in response to environmental cues. This is now well documented

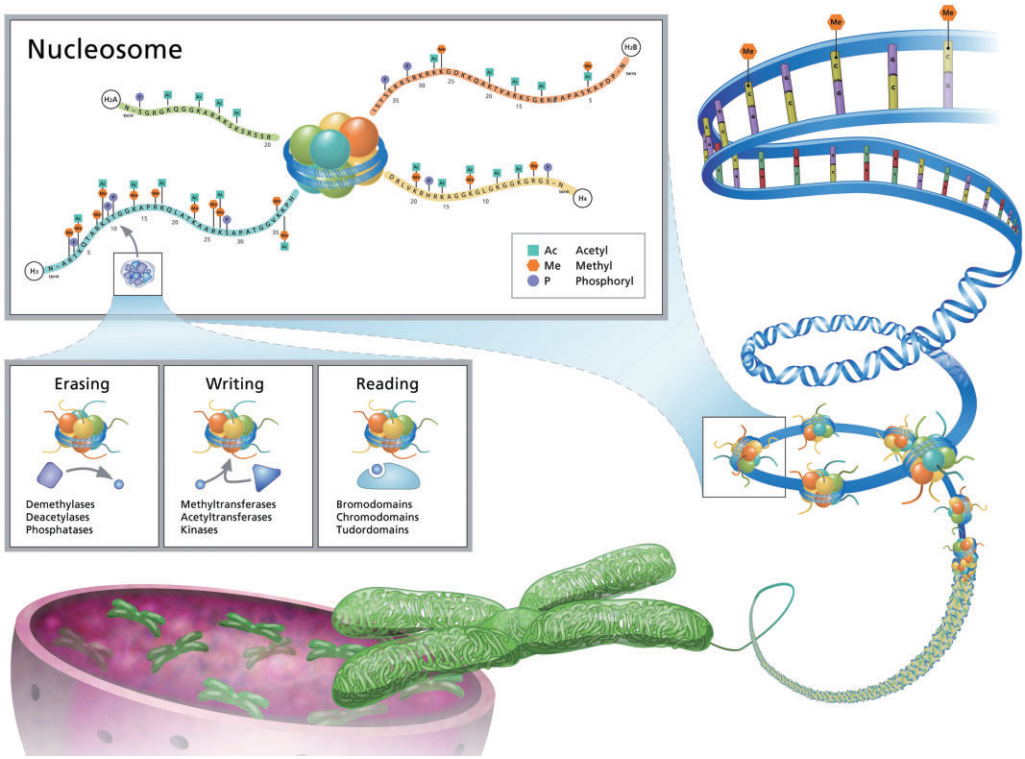


Figure 1

Histone modification in the regulation of chromatin structure. Nucleosomes, which represent the core subunits of chromatin, are subject to numerous modifications that influence chromatin structure and gene expression. Many different amino acids in the core histones can be altered by enzymes termed epigenetic 'writers' that generate various post-translational modifications, such as acetylation, methylation and phosporylation. These marks can be removed by 'erasers' and are recognised by 'reader' proteins which contain domains capable of specific recognition of the modified peptide sequences.

for fundamental biological processes such as the differentiation of activated T-cells into effector subsets, where the capacity of the cells to produce different cytokines is controlled by epigenetic modifications in cytokine gene regions that are induced by specific conditions of T-cell activation (Kanno *et al.*, 2012). However, there is also growing evidence that aberrant epigenetic states are associated with a range of pathologies, including inflammatory, neuropsychiatric, cardiovascular, and metabolic diseases and cancer. The identification of the proteins responsible for writing, erasing and reading chromatin modifications has opened up a new area of drug discovery, with the hope of being able to reset abnormal epigenetic landscapes back to normal and thus provide lasting health benefits in these diseases. In this review, we discuss early progress in developing small molecule pharmacological modulators of epigenetic targets, focusing on a subset of histone writers, erasers and readers where understanding of biological relevance and chemical tractability is most advanced.

Lysine acetyltransferases

Multiple lysine residues in each of the core histones are subject to acetylation. Histone acetylation is mediated by a family of enzymes, the lysine acetyltransferases (KATs), which utilize acetyl CoA as a cofactor to catalyse the transfer of an acetyl group to the ε-amino group of lysine side chains (Roth *et al.*, 2001). At least 17 mammalian KATs able to acetylate histones have been identified, although the relatively low sequence homology among protein acetyltransferases suggests that many such enzymes could yet be discovered (Yuan and Marmorstein, 2012). These enzymes have traditionally been grouped into families based on similarity in the sequence of their catalytic domains and biochemical mechanism of acetyl transfer, and are listed utilizing the simplified nomenclature proposed by Allis *et al.* (Sterner and Berger, 2000; Roth *et al.*, 2001; Allis *et al.*, 2007; Furdas *et al.*, 2012) in Table 1. Different KATs have been shown to preferentially actetylate distinct lysine residues in histones, although considerable overlap appears to exist.

Two things are worth noting with respect to the designation of these enzymes as histone acetyltransferases. First, several of these KATs are known to also acetylate non-histone proteins, which may make an important contribution to their function; this idea is supported by the detection of lysine acetylation in nearly 2000 proteins involved in many key cellular processes (Choudhary *et al.*, 2009). Second, in some cases, enzymes have only been shown directly to acetylate histones *in vitro*, and their ability to do so in a cellular context remains unknown.

Acetylation of histone tails has long been associated with active gene transcription (Marushige, 1976). This is linked to

Table 1

Lysine acetyltransferases

Family	Proposed symbol*	Synonyms and other symbols	Uni-ProtKB/Swiss-Prot assession number
GNAT	KAT2A	GCN5, GCN5L2, HGCN5, PCAF-b	Q92830
	KAT2B	PCAF, P/CAF	Q92831
	KAT9	ELP3, FLJ10422	Q9H9T3
P300/CBP	KAT3A	CBP, CREBBP, RSTS, RTS	Q92793
	KAT3B	P300, EP300	Q09472
MYST	KAT5	TIP60, PLIP, HTATIP, cPLA2, HTATIP1, ESA1, ZC2HC5	Q92993
	KAT6A	MOZ, MYST3ZNF220, RUNXBP2, ZC2HC6A	Q92794
	KAT6B	MORF, MYST4, querkopf, qkf, MOZ2, ZC2HC7	Q8WYB5
	KAT7	HBO1, MYST2, HBOA, ZC2HC7	O95251
	KAT8	hMOF, MYST1, MOF, FLJ14040, ZC2HC8	Q9H7Z6
Transcription factor-related	KAT4	TAF1, TAF2A, BA2R, CCG1, CCGS, DYT3, NSCL2, TAFII250, DYT3/TAF1	P21675
	KAT12	TFIIIC90, GTF3C4	Q9UKN8
NR co-activators	KAT13A	SRC1, NCOA1, F-SRC-1, NCoA-1, RIP160, bHLHe74	Q15788
	KAT13B	AIB1, ACTR, SRC3, NCOA3, RAC3, P/CIP, TRAM-1, cAGH16, TNRC16, bHLHe42, SRC-3	Q9Y6Q9
	KAT13C	P160, NCOA2, TIF2, GRIP1, NCoA-2, bHLHe75	Q15596
	KAT13D	CLOCK, KIAA0334	O15516
Type B (cytoplasmic)	KAT1	HAT1/HATB	O14929

*Proposed symbols are those suggested by Allis *et al.* (2007). A list of the proposed lysine acetyltransferase names and symbols that have been approved by the HUGO Gene Nomenclature Committee (HGNC) can be found at http://www.genenames.org/genefamilies/kdm-kat-kmt#KAT.

the ability of acetylation to generate a more open chromatin structure (Hong *et al.*, 1993), and to the recruitment of specific reader proteins able to bind to acetylated histones (see below). Not surprisingly, therefore, many histone acetyltransferases are known to function in transcriptional activation. However, histone acetylation is also known to influence many other processes, including cell cycle progression, chromosome dynamics, DNA recombination, DNA repair and cellular apoptosis, indicating that acetylation plays a central role in regulating chromatin-related functions (Khan and Khan, 2010).

Misregulation of histone acetyltransferase activity has been linked to many different pathogenic states, including multiple cancers, neurodegenerative disorders, plus metabolic, respiratory, inflammatory and cardiovascular diseases; as such, KATs represent attractive targets for drug development (Adcock and Lee, 2006; Avvakumov and Cote, 2007; Grabiec *et al.*, 2008; Ghizzoni *et al.*, 2011; Iyer *et al.*, 2011; Pirooznia and Elefant, 2013). Several different types of KAT inhibitors have been described (Table 2). Peptide-based bisubstrate inhibitors, in which CoA is covalently linked to the ζ nitrogen of the target lysine within histone peptides, act as structural mimics and can show sub-micromolar potency and selectivity between KAT subfamilies (Lau *et al.*, 2000). However, since these inhibitors lack cellular permeability, they have limited use for evaluation of the cellular function of the KATs. Natural products isolated from a number of different plants, as well as their synthetic derivatives, have been shown to possess KAT inhibitory activity (Balasubramanyam *et al.*, 2003; 2004a,b; Choi *et al.*, 2009; Ravindra *et al.*, 2009). In addition, small molecule KAT inhibitors have been identified by high-throughput biochemical and computational (virtual) screens (Gorsuch *et al.*, 2009; Bowers *et al.*, 2010). One compound identified from a virtual screen, C646, was reported to be selective for KAT3A/3B over other KATs and was shown to inhibit the growth of tumour cells *in vitro* (Bowers *et al.*, 2010).

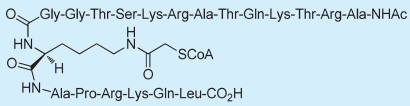
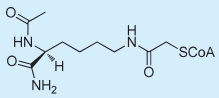
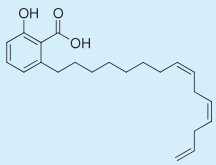
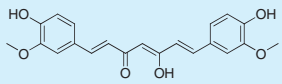
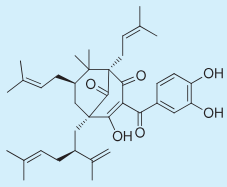
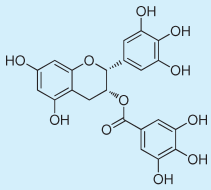
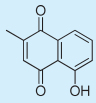
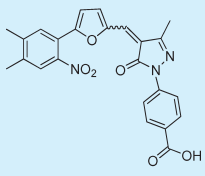
Overall, however, the development of therapeutic KAT inhibitors is at an early stage, and current compounds are suboptimal in a number of ways, because they generally lack potency and selectivity. Nevertheless, one natural product KAT inhibitor, curcumin, has entered into clinical trials for a number of diseases, including Alzheimer's disease, rheumatoid arthritis, cystic fibrosis and psoriasis; there are currently 85 studies at different stages in ClincalTrials.gov that list curcumin or related compounds. As this compound is known to affect a number of other epigenetic and non-epigenetic targets, including DNMT I and lysine deacetylases (KDACs), linking potential efficacy with effects on KATs will not be straightforward.

Lysine deacetylases

The reversal of histone acetylation is mediated by members of the KDAC family, which comprises 18 enzymes that can be

Table 2

Examples of KAT inhibitors

Compound	Structure	Potency (μM)	Known KAT target	Source	Reference
H3-CoA-20		0.5	KAT2B	Peptide-based bisubstrate inhibitors	(Lau *et al.*, 2000)
Lys-CoA		0.5	KAT3B	Peptide-based bisubstrate inhibitors	(Lau *et al.*, 2000)
Anacardic acid		5–8.5	KAT2B, KAT3B	Cashew nut shell	(Balasubramanyam *et al.*, 2003)
Curcumin		25	KAT3A, KAT3B	Turmeric (spice)	(Balasubramanyam *et al.*, 2004b)
Garcinol		5–7	KAT2B, KAT3B	Garcinia indica fruit rind	(Balasubramanyam *et al.*, 2004a)
Epigallocatechin-3-gallate (EGCG)		30–50	Pan-KAT	Green tea	(Choi *et al.*, 2009)
Plumbagin (RTK1)		20–50	KAT3A, KAT3B, KAT2B	Plumbago rosea root	(Ravindra *et al.*, 2009)
C646		5	KAT3A, KAT3B	Small molecule virtual screen	(Bowers *et al.*, 2010)

divided into four classes based on their homology to the yeast orthologues Rpd3, HdaI and Sir2 (Gregoretti *et al.*, 2004) (Table 3). The class I, II and IV enzymes are termed histone deacetylases (HDACs), while the class III deacetylases are named sirtuins (SIRTs). The seven sirtuins (SIRT1–7) share a conserved catalytic core domain and use NAD^+ as an essential cofactor, while HDACs contain a Zn^{2+} ion in their active site. Notwithstanding the nomenclature, several sirtuins do function as HDACs, while at least one HDAC, HDAC6, appears to be almost exclusively localized to the cytoplasm and may not deacetylate histones *in vivo* (Verdel *et al.*, 2000; Zhang *et al.*, 2003; Martinez-Redondo and Vaquero, 2013). In addition to deacetylase activity, some SIRT proteins also possess other enzymatic activities. Thus, while SIRT1–3 and SIRT7 act primarily as KDACs, SIRT4 is an ADP-ribosyltransferase, SIRT5 is a deacetylase, demalonylase, and desuccinylase, and SIRT6 is an ADP-ribosyltransferase and a deacetylase (Roth and Chen, 2013). As for the KATs, KDACs can deacetylate many histone and non-histone proteins.

In accordance with the key role that acetylation plays in the regulation of chromatin structure, as well as the expansive function of lysine acetylation in protein networks and cellular signalling pathways (Choudhary *et al.*, 2009), HDACs have been implicated in the regulation of gene expression

Table 3

Lysine deacetylases

Class	Symbol	Cellular localization	Uni-ProtKB/Swiss-Prot assession number
I (Rpd3)	HDAC1	Nucleus	Q13547
	HDAC2	Nucleus	Q92769
	HDAC3	Nucleus	O15379
	HDAC8	Nucleus/cytoplasm	Q9BY41
IIa (Hda1)	HDAC4	Nucleus/cytoplasm	P56524
	HDAC5	Nucleus/cytoplasm	Q9UQL6
	HDAC7	Nucleus/cytoplasm	Q8WUI4
	HDAC9	Nucleus/cytoplasm	Q9UKV0
IIb (Hda1)	HDAC6	Cytoplasm	Q9UBN7
	HDAC10	Cytoplasm	Q969S8
III (Sir)	SIRT1	Nucleus/cytoplasm	Q96EB6
	SIRT2	Nucleus/cytoplasm	Q8IXJ6
	SIRT3	Mitochondria	Q9NTG7
	SIRT4	Mitochondria	Q9Y6E7
	SIRT5	Mitochondria	Q9NXA8
	SIRT6	Nucleus	Q8N6T7
	SIRT7	Nucleolus	Q9NRC8
IV (Rpd3/Hda1)	HDAC11	Nucleus/cytoplasm	Q96DB2

and in the control of many important cellular processes such as proliferation, DNA repair and apoptosis (Lahue and Frizzell, 2012; Li *et al.*, 2012; Matthews *et al.*, 2012; Yao and Rahman, 2012; Joshi *et al.*, 2013; Ververis *et al.*, 2013). Moreover, dysregulation of HDACs has been proposed to contribute to a variety of diseases including cancer, interstitial fibrosis, autoimmune and inflammatory diseases, and metabolic disorders (Tang *et al.*, 2013a). For this reason, considerable effort has gone into the development of HDAC inhibitors.

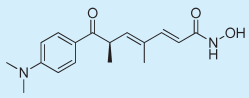
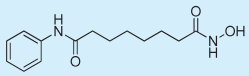
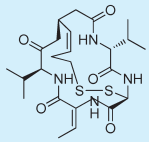
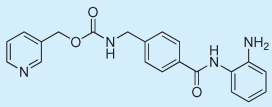
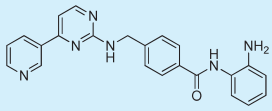
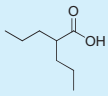
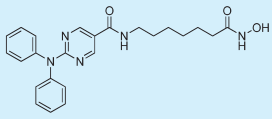
Most of the HDAC inhibitors generated to date show activity against multiple family members. Among these, there is a range in the breadth of activity (Table 4) – from those that inhibit essentially all HDACs [e.g. trichostatin A and suberoylanilide hydroxamic acid (SAHA)], to some that are active against class I and IIa HDACs (e.g. butyric acid, valproic acid), to others that appear more selective to class I HDACs (e.g. romidepsin, MS-275) (Khan and La Thangue, 2012) or class IIa HDACs (Lobera *et al.*, 2013). Experiments using such non-selective HDAC compounds have provided much of the evidence that HDAC inhibition could be effective in a range of therapeutic areas (Grabiec *et al.*, 2011; Tang *et al.*, 2013a). More recently, inhibitors with selectivity for HDAC1, HDAC3, HDAC6 and HDAC8 have been described (Hu *et al.*, 2003; Schlimme *et al.*, 2011; Cantley and Haynes, 2013; Jochems *et al.*, 2013). Although there is currently limited understanding of the biological implications of targeting individual family members, HDAC6-selective compounds have been reported to inhibit cancer cell proliferation *in vitro* and in xenograft models *in vivo* and to exert antidepressant activity in a mouse model (Schlimme *et al.*, 2011; Santo *et al.*, 2012; Jochems *et al.*, 2013). The latter activity was shown to be associated with increased acetylation of the HDAC6 target α-tubulin rather than histone acetylation (Jochems *et al.*, 2013).

The ability of HDAC inhibitors to induce death, cytostasis or differentiation of tumour cells in preclinical models, combined with evidence of HDAC up-regulation in a variety of cancers, has provided a strong rationale for progressing such compounds into clinical trials for oncology (Giannini *et al.*, 2012). Presently, there are 394 trials that include the term 'HDAC inhibitor' recorded in ClinicalTrials.gov, with the vast majority of these being in cancer. A large number of trials in multiple solid and haematological tumour types are in progress, with the most promising results obtained so far being observed when HDAC inhibitors were combined with other agents such as proteasome inhibitors (Khan and La Thangue, 2012; Qiu *et al.*, 2013; Richardson *et al.*, 2013; Ververis *et al.*, 2013). Currently, there are two HDAC inhibitors that have received approval from the US FDA for the treatment of cutaneous T-cell lymphoma: vorinostat (SAHA, Zolinza®; Merck & Co., Inc., Whitehouse Station, NJ, USA) and depsipeptide (romidepsin, Istodax, Celgene Corporation, Summit, NJ, USA).

Although broadly active HDAC inhibitors are being tested in the vast majority of trials, one HDAC1-selective inhibitor (MGCD0103) has progressed into clinical trials in haematological tumours, and a HDAC6-selective inhibitor is being evaluated as a monotherapy and also in combination with other treatments in patients with relapsed or relapsed/refractory multiple myeloma (NCT01323751, NCT01583283). By selectively targeting HDAC subfamily

Table 4

Examples of HDAC inhibitors

Name	Structure	Potency	HDAC specificity	Clinical trial (cancer)
TSA (trichostatin A)		1.8 nM (HDAC5)	Class I, II, IV	–
Vorinistat (SAHA, suberoylanilide hydroxamic acid)		10 nM (HDAC1,2,3,8,9)	Class I, II, IV	FDA approved (2006) Phase II, III
Romidepsin (FK228)		36–47 nM (HDAC1,2)	Class I	FDA approved (2009) Phase I, II
Entinostat (MS-275)		500 nM (HDAC1,2,3,9)	Class I	Phase II
Mocetinostat (MGCD0103)		0.15–1.66 μM (HDAC1,2,3)	HDAC1	Phase I, II
Butyric acid		mM	Class I, II	Phase II
Valproic acid		0.7–20 mM (HDAC1,2,3)	Class I, II	Phase I, II, III
ACY-1215 (rocilinostat)		5 nM	HDAC6	Phase I/II

members, it is hoped that there will be an opportunity to reduce toxic side effects associated with broadly inhibiting lysine deacetylation and hence for an improved therapeutic treatment window. Another strategy being evaluated in this respect is to design compounds that will preferentially accumulate in specific cell types. This is the basis behind the HDAC inhibitor CHR-2845, a cell-permeant ester that is metabolized to give an active acid which selectively accumulates in monocytes and macrophages. This compound has been evaluated for tolerability in patients with advanced haematological malignancies (NCT00820508), and it is hoped that this approach will be effective in haematological malignancies involving cells of the monocyte lineage.

Reflecting the broad biological effects of HDAC inhibition in preclinical studies, HDAC inhibitors have also entered into trials in other therapeutic areas, including graft versus host disease (NCT01111526), sickle cell disease (NCT01000155), Huntington's disease (NCT00212316), Rubinstein–Taybi syndrome (NCT01619644) and human immunodeficiency virus (HIV; NCT01680094). In the latter study, the aim is to evaluate the ability of the compound to reactivate HIV transcription in latently infected $CD4^+$ T-cells, which would form part of an approach to deplete the latent pool of virus (in combination with anti-retroviral therapy). Evidence that HDAC inhibitors can induce such HIV reactivation has come from studies involving *ex vivo* compound treatment of cells from HIV-infected individuals, although initial small scale clinical trials have yielded conflicting results concerning the combined effects of HDAC inhibition and anti-retroviral therapy on viral load (Margolis, 2011).

Much of the interest in the SIRT family of KDACs has focused on the function of these enzymes in metabolic, oxidative/genotoxic, and oncogenic stress responses, where their deacetylation of non-histone substrates may play a predominant role. In view of the protective role of SIRTs in these processes, a major therapeutic focus has been on the development of SIRT activators for the treatment of ageing-associated pathologies, including type II diabetes, cardiovascular disease and neurodegeneration (Hall *et al.*, 2013). SIRT1 activation has also been implicated in suppressing the immune response, leading to an interest in developing SIRT activators for treatment of autoimmune and inflammatory diseases (Kong *et al.*, 2013). In cancer, the role of SIRT proteins is complex, with evidence for SIRTs playing roles in both promoting and suppressing tumourigenesis (Roth and Chen, 2013).

Small molecules capable of SIRT1 activation have been identified, including the weakly active polyphenol compound resveratrol, a component of red wine, and a series of compounds structurally unrelated to resveratrol with 1000-fold greater potency against SIRT1 (Dittenhafer-Reed *et al.*, 2011). SIRT1 activators became the subject of controversy when it was shown that these compounds activated enzyme activity towards fluorescently labelled peptide substrates but not their unlabelled counterparts, suggesting a possible assay artefact (Kaeberlein *et al.*, 2005; Pacholec *et al.*, 2010). However, an intriguing explanation for these observations was subsequently provided when it was shown that the bulky hydrophobic fluorophore tag on the assay peptide mimicked hydrophobic amino acids present in a subset of natural SIRT1 protein substrates that are selectively subject to increased deacetylation after SIRT1 activation (Hubbard *et al.*, 2013; Lakshminarasimhan *et al.*, 2013). In addition, a single amino acid substitution outside of the catalytic site in SIRT1 was found to abolish activation of the enzyme as well as the cellular effects mediated by SIRT1 activators, demonstrating that SIRT1 is indeed the target of these compounds.

Trials of resveratrol in obese humans produced mild improvements in a number of different clinical parameters including systolic blood pressure, circulating cytokines, intrahepatic fat content, intramyocellular lipid content, and muscle mitochondrial oxidative phosphorylation capacity (Timmers *et al.*, 2011). However, because resveratrol has known activity against a number of substrates besides SIRT1, including AMP-activated kinase, a fuel-sensing enzyme that is responsive to decreases in cellular energy status, the mechanism by which resveratrol mediates these effects, remains a subject of debate. Early phase clinical trials investigating higher potency SIRT1 activators (SRT2104, SRT2379) in both metabolic diseases and inflammation have been conducted (Libri *et al.*, 2012; Hoffmann *et al.*, 2013). Published results for SRT2104 indicate that this compound appears to be safe and well tolerated and associated with an improved lipid profile (Libri *et al.*, 2012).

Lysine methyltransferases

A large number of enzymes capable of transferring methyl groups to lysine residues have been described. The 24 human lysine methyltransferases (KMTs) categorized by Allis *et al.* (2007) are listed in Table 5. All KMTs except one [KMT4/Dot1L, a unique KMT, which belongs to the class I methyltransferase family (Min *et al.*, 2003)] contain a catalytic domain of approximately 130 amino acids, referred to as the SET domain; both SET domain-containing enzymes and KMT4 use S-adenosyl methionine (SAM) as the methyl donor. Based on a systematic screen for SET domains in the human genome, 51 putative KMTs have been identified, although the enzymatic activity of many of these is yet to be investigated (Copeland *et al.*, 2009; Richon *et al.*, 2011). KMTs exhibit selectivity for both the lysine residue they can modify and the degree to which that lysine residue is methylated. Thus, while lysine residues can only accept a single acetyl group, lysines can be mono-, di- or trimethylated. The site specificity of lysine methylation is determined by recognition of amino acid residues flanking the target lysine, whereas particular amino acids within the lysine-binding channel of the KMTs play an important role in dictating the methylation multiplicity of the SET domain (Qian and Zhou, 2006).

In contrast to histone acetylation, histone methylation does not alter the charge of the histone tail, but instead influences its basicity and hydrophobicity (Migliori *et al.*, 2010). As for acetylation, methylated lysines also serve as recognition marks for a large family of methyl reader proteins, which show selectivity for both the specific lysine residue modified and the degree of methylation. As a consequence, different types of lysine methylation are associated with divergent functions in the regulation of gene expression. For example, trimethylation of H3K4 (H3K4me3) is commonly found near the transcription start site of genes that are actively expressed or poised for expression, whereas H3K27me3 is a mark that is associated with genes for which expression is suppressed (Kouzarides, 2007). Conversely, the presence of H3K4me1 is tightly correlated with the position of gene enhancers, which are non-coding regions of DNA that promote the expression of genes that are often located a considerable distance away (Heintzman *et al.*, 2009).

In addition to the histone tail, the residues within the core of the nucleosome can also be modified by methylation. For instance, H3K79 is subject to methylation by KMT4, and methylated H3K79 is associated with active gene transcription (Feng *et al.*, 2002; Steger *et al.*, 2008; Kim *et al.*, 2013). Lysine 20 is the only well-characterized methylation site on histone H4, with methylated H4K20 being linked with transcriptional repression and a number of biological processes, including the DNA damage response, mitotic condensation and DNA replication (Jorgensen *et al.*, 2013); the transition from H4K20me1 to me2 and me3 was recently associated with cellular quiescence (Evertts *et al.*, 2013).

Misregulation of KMTs has been linked to a variety of human diseases, and therapeutic interest in the development of KMT inhibitors is particularly strong in cancer, where the pathological involvement of KMT overexpression, mutation and translocation has been shown (Copeland *et al.*, 2009). One interesting example of a potential cancer target is KMT4, which is implicated in leukaemias involving chromosomal translocation of another KMT, KMT2A (MLL1). In such cancers, KMT4 is recruited into the transcriptional complex via MLL-fusion partners, and aberrant methylation of H3K79 is considered to play a causative role in disease (Steger *et al.*, 2008; Bernt *et al.*, 2011). Another target of major interest is KMT6, which is a key mediator of di- and trimethylation of H3K27 and is overexpressed in many types of cancers (Chang and Hung, 2012).

In recent years, small molecule KMT inhibitors have been developed that are directed at either the SAM or the substrate site of the enzymes (Wigle and Copeland, 2013). Inhibitors have been reported for various KMTs, including KMT4, KMT6, KMT1C/D, KMT3C and KMT5A (Table 6). Compounds targeting KMT4, KMT1C/D and KMT6 have shown promising efficacy in preclinical tumour models (Daigle *et al.*, 2011; 2013; Yuan and Marmorstein, 2012; Yuan *et al.*, 2012; Knutson *et al.*, 2013; Liu *et al.*, 2013), strengthening the rationale for targeting these enzymes in cancer. Building on this rationale, the KMT6 inhibitor E7438 is currently being trialled in patients with advanced solid tumours or with B-cell lymphomas (Knutson *et al.*, 2013) (NCT01897571) while the

Table 5

Lysine methyltransferases

Proposed symbol*	Synonyms and other symbols	Histone substrate specificity	Uni-ProtKB/Swiss-Prot assession number
KMT1A	SUV39H1, SUV39H	H3K9me2/3	O43463
KMT1B	SUV39H2, FLJ23414	H3K9me2/3	Q9H5I1
KMT1C	G9a, EHMT2, C6orf30, BAT8, Em:AF134726.3, NG36/G9a	H3K9me1/2, H1K26me1/2	Q96KQ7
KMT1D	GLP, EHMT1, Eu-HMTase1, FLJ12879, KIAA1876, bA188C12.1	H3K9me1/2	Q9H9B1
KMT1E	ESET, SETDB1, KG1T, KIAA0067, TDRD21	H3K9me2/3	Q15047
KMT1F	SETDB2, CLL8, C13orf4, CLLD8	H3K9me2/3	Q96T68
KMT2A	MLL1, TRX1, HRX, ALL-1, HTRX1, CXXC7, MLL1A, MLL	H3K4me1/2/3	Q03164
KMT2B	MLL2, KIAA0304, TRX2, HRX2, WBP7, MLL1B, MLL4	H3K4me1/2/3	Q9UMN6
KMT2C	MLL3, KIAA1506, HALR	H3K4me1/2/3	Q8NEZ4
KMT2D	MLL4, TNRC21, MLL2, ALR, CAGL114	H3K4me1/2/3	O14686
KMT2E	MLL5, HDCMC04P	H3K4me1/2/3	Q8IZD2
KMT2F	hSET1A , SETD1A, KIAA0339, Set1	H3K4me1/2/3	O15047
KMT2G	hSET1B, SETD1B, KIAA1076, Set1B	H3K4me1/2/3	Q9UPS6
KMT2H	ASH1, ASH1L, huASH1, ASH1L1	H3K4me1/2/3, H3K9me1/2/3, H4K20me1/2/3	Q9NR48
KMT3A	SET2, SETD2, HYPB, HIF-1, KIAA1732, FLJ23184	H3K36me3	Q9BYW2
KMT3B	NSD1, STO, ARA267, FLJ22263	H3K36me1/2, H4K20me1/2	Q96L73
KMT3C	SYMD2, HSKM-B, ZMYND14	H3K36me1/2	Q9NRG4
KMT3D	SMYD1, BOP, ZMYND22	Unknown	Q8NB12
KMT3E	SMYD3, ZNFN3A1, ZMYND1	H3K4me2/3	Q9H7B4
KMT4	DOT1L , KIAA1814, DOT1	H3K79me1/2/3	Q8TEK3
KMT5A	Pr-SET7, SETD8, SET07, SET8	H4K20me1	Q9NQR1
KMT5B	SUV420H1, CGI-85	H4K20me2/3	Q4FZB7
KMT5C	SUV420H2, MGC2705	H4K20me2/3	Q86Y97
KMT6A	EZH2, EZH1, ENX-1, KMT6	H3K27me1/2/3, H1K26me2/3	Q15910
KMT6B	EZH1, KIAA0388	H3K27me1/2/3	Q92800
KMT7	SET7/9, KIAA1717, SET7, Set9	H3K4me2	Q8WTS6
KMT8	PRDM2, RIZ1, RIZ, RIZ2, MTB-ZF, HUMHOXY1	H3K9me1/2/3	Q13029

*Proposed symbols are those suggested by Allis *et al.* (2007). A list of the proposed lysine methyltransferase names and symbols that have been approved by the HUGO Gene Nomenclature Committee (HGNC) can be found at http://www.genenames.org/genefamilies/kdm-kat-kmt#KMT.

KMT4 inhibitor EPZ-5676 has recently entered phase I studies in patients with advanced haematological malignancies (NCT01684150) (Copeland, 2013; Daigle *et al.*, 2013).

Lysine demethylases

Lysine-specific demethylases (KDMs) capable of removing methyl groups from histones are classified into two families (Table 7). One family is comprised of KDM1A (LSD1) and the closely related KDM1B (LSD2), demethylate lysines, utilizing a flavin adenine dinucleotide (FAD)-dependent oxidation mechanism (Shi *et al.*, 2004; Karytinos *et al.*, 2009). KDM1A and KDM1B are able to demethylate mono- and dimethylated lysines, but cannot remove the methyl group from trimethylated lysine because of the dependence of the demethylation mechanism on a protonated amine (Hou and Yu, 2010). The second much larger family of histone demethylases is formed by the jumonji C-domain (Jmj)-containing enzymes, which catalyse lysine demethylation through a hydroxylation pathway utilizing Fe^{2+} and α-ketoglutarate (α-KG) as cofactors (Tsukada *et al.*, 2006). Unlike KDM1A and KDM1B, the Jmj family of enzymes does not depend on a protonated amine and therefore can demethylate trimethylated lysines.

As a group, the KDMs have been reported to erase a range of methyl marks on histones (Table 7). Enzymes show preferences for both the degree of lysine methylation and the histone sequence, although promiscuity with respect to the latter is exhibited by some KDMs. This promiscuity can be attributed to the presence of similar amino acids flanking the methylated lysine in distinct peptides or to a dominant role of the peptide backbone, rather than the side chains, in

Table 6

Recent examples of lysine methyltransferase inhibitors

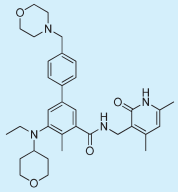
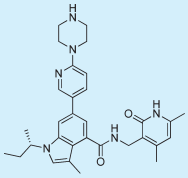
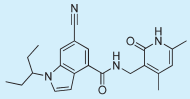
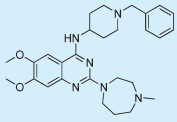
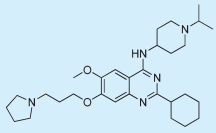
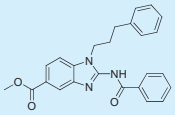
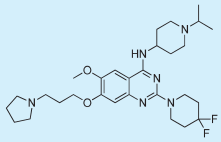
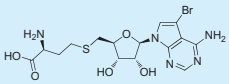
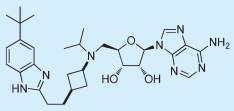
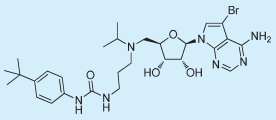
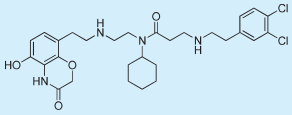
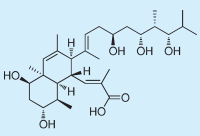
Name	Structure	Potency	KMT specificity	Reference
EPZ-6438		2.5 nM	MT6	(Knutson *et al.*, 2013)
GSK126		0.5–3 nM	KMT6	(McCabe *et al.*, 2012)
EI1		9.4 nM	KMT6	(Qi *et al.*, 2012)
BIX-01294		2.7 μM	KMT1C/D	(Kubicek *et al.*, 2007; Lu *et al.*, 2013)
UNC0638		<15 nM	KMT1C/D	(Vedadi *et al.*, 2011)
BRD4770		6.3 μM	KMT1C/D	(Yuan and Marmorstein, 2012; Yuan *et al.*, 2012)
UNC0642		<2.5 nM	KMT1C/D	(Liu *et al.*, 2013)
Bromo-deaza-SAH		77 nM	KMT4	(Yu *et al.*, 2013)
EPZ-5676		80 pM	KMT4	(Daigle *et al.*, 2011)
SGC0946		0.3 nM	KMT4	(Yu *et al.*, 2012)
AZ505		0.12 μM	KMT3C	(Ferguson *et al.*, 2012)
Nahuoic acid		2 μM	KMT5A	(Williams *et al.*, 2013)

Table 7

Lysine demethylases

Proposed symbol*	Synonyms and other symbols	Reported histone substrate specificity	Uni-ProtKB/ Swiss-Prot assession number
KDM1A	LSD1, AOF2, BHC, KIAA0601, BHC110,	H3K4me1/2, H3K9me1/2	O60341
KDM1B	LSD2, AOF1, C6orf193, FLJ34109, FLJ33898, dJ298J15.2, bA204B7.3, FLJ43328	H3K4me1/2	Q8NB78
KDM2A	JHDM1A, FBXL11, CXXC8, FBL7, KIAA1004, FBL11, LILINA, DKFZP434M1735, FLJ00115	H3K4me3, H3K36me1/2	Q9Y2K7
KDM2B	JHDM1B, FBXL10, CXXC2, FBL10, PCCX2	H3K36me1/2	Q8NHM5
KDM3A	JMJD1A , JHDM2A, JMJD1, KIAA0742, TSGA	H3K9me1/2	Q9Y4C1
KDM3B	JMJD1B, JHDM2B, C5orf7, KIAA1082NET22	H3K9me1/2	Q7LBC6
KDM3C	JMJD1C, JHDM2C	H3K9me1/2	Q15652
KDM4A	JMJD2A , JHDM3A, JMJD2, KIAA0677, TDRD14A	H3K9me2/3, H3K36me2/3, H1K26me3	O75164
KDM4B	JHDM3B, JMJD2B, KIAA0876, TDRD14B	H3K9me2/3, H3K36me2/3, H1K26me3	O94953
KDM4C	JMJD2C, GASC1, JHDM3C, KIAA0780, TDRD14C	H3K9me2/3, H3K36me2/3, H1K26me3	Q9H3R0
KDM4D	JMJD2D, JHDM3D, FLJ10251	H3K9me2/3, H1K26me2/3	Q6B0I6
KDM4E	JMJD2E, KDM4DL	H3K9me2/3	B2RXH2
KDM5A	JARID1A, RBBP2, RBP2	H3K4me1/2/3	P29375
KDM5B	JARID1B, PLU-1, RBBP2H1, RBBP2H1A, CT31	H3K4me1/2/3	Q9UGL1
KDM5C	JARID1C, SMCX, DXS1272E, XE169, MRX13	H3K4me1/2/3	P41229
KDM5D	JARID1D, SMCY, HY, HYA, KIAA0234	H3K4me2/3	Q9BY66
KDM6A	UTX	H3K27me2/3	O15550
KDM6B	JMJD3, KIAA0346	H3K27me2/3	O15054
KDM7A	JHDM1D, KIAA1718	H3K9me1/me2, H3K27me1/me2	Q6ZMT4
KDM7B	PHF8, JHDM1F, KIAA1111, ZNF422, DKFZp686E0868	H3K9me2/me1, H3K27me2, H3K36me2	Q9UPP1
KDM7C	PHF2, JHDM1E, GRC5, KIAA0662, MGC176680, CENP-35	H3K9me2	O75151
KDM8	JMJD5, FLJ13798	H3K36me2	Q8N371

*Proposed symbols are those suggested by Allis *et al.* (2007). A list of the proposed lysine demethylase names and symbols that have been approved by the HUGO Gene Nomenclature Committee (HGNC) can be found at http://www.genenames.org/genefamilies/kdm-kat-kmt#KDM.

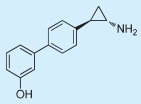
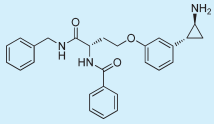
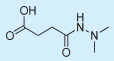
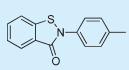
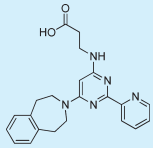
recognition by the enzyme (Hou and Yu, 2010). Importantly, while substrate specificity is often defined *in vitro* using isolated catalytic domains, the actual lysine residues targeted *in vivo* may be confined by other mechanisms. For example, although KDM7B can demethylate H3K9me2/me1, H3K27me2 and H3K36me2 *in vitro*, the intact protein contains a plant homeo domain finger that binds to H3K4me3, directing the catalytic domain towards H3K9me2 (Horton *et al.*, 2009). In addition to regulation by other domains within the same protein, KDMs are typically found within multi-protein complexes that will greatly influence their targeting to chromatin. As for other histone-modifying enzymes, the KDMs can also act on non-histone substrates, which may play a key role in their function. For example, KDM1A has been shown to demethylate key cellular targets such as p53, DNMT 1, STAT3, E2F1 and MYPT1 (Huang *et al.*, 2007a; Wang *et al.*, 2009a; Kontaki and Talianidis, 2010; Yang *et al.*, 2010; Cho *et al.*, 2011).

Given the correlation between particular methyl marks and the transcriptional state of genes, it is expected that the activity of specific KDMs will be linked to gene activation or repression, depending on the KDM substrate. Notably, however, some KDMs such as KDM1A possess the capacity to erase marks associated with both active (H3K4me2) and silent (H3K9me2) genes. In addition, there is considerable apparent redundancy in substrate specificity, with multiple KDMs able to erase the same marks (Table 7). Thus, the functions of KDMs within cells are likely to be determined by multiple factors such as KDM expression level, enzymatic activity, and targeting to specific sites within the genome in the context of particular cells and specific environmental signals. The development of potent and selective inhibitors against members of the family combined with sophisticated epigenetic mapping of functional outcomes (mark and gene transcription level) will be essential to define these in a cellular context.

A major focus of therapeutic interest in KDMs is in oncology, as mutations and aberrant expression of KDMs have been linked to various cancers (Hojfeldt *et al.*, 2013). For example, KDM1A is reported to be overexpressed in a number of different cancers, including neuroblastoma, breast cancer,

Table 8

Examples of KDM inhibitors

Name	KDM specificity/bias	Structure	Potency (μM)	Reference
OG-L002	KDM1A		0.02	(Liang *et al.*, 2013)
NCL-1	KDM1A		1.6	(Ogasawara *et al.*, 2011)
Daminozide	KDM2/7		1.5–2.1	(Rose *et al.*, 2012)
PBIT	KDM5B		3	(Sayegh *et al.*, 2013)
GSK-J1	KDM6A/B		5	(Kruidenier *et al.*, 2012)

lung cancer, prostate cancer and bladder cancer (Schulte *et al.*, 2009; Chi *et al.*, 2010; Lim *et al.*, 2010; Hayami *et al.*, 2011). The other FAD-dependent KDM, KDM2B, has also been linked to cancer, with amplification and high expression observed in urothelial carcinoma (Heidenblad *et al.*, 2008). Likewise, Jmj KDMs including KDM2B, KDM4A, KDM4B, KDM4C, KDM5B and KDM7C have been shown to be overexpressed in breast, colorectal, lung, prostate, bladder and other tumours; the functional significance of KDM4C overexpression is further suggested by the presence of the KDM4C gene within an amplified region of a chromosome in multiple cancers (Xiang *et al.*, 2007; Couvelard *et al.*, 2008; Roesch *et al.*, 2010; He *et al.*, 2011a; Berry and Janknecht, 2013; Kogure *et al.*, 2013; Tzatsos *et al.*, 2013). Notably, both increased and decreased expressions of KDMs may be associated with cancer, suggesting a key role for precise control of lysine methylation in maintaining cellular homeostasis. For example, while overexpression of KDM6A is associated with breast cancer and renal cell carcinoma, inactivating mutations in KDM6A are also found in multiple cancer types (van Haaften *et al.*, 2009; Dalgliesh *et al.*, 2010; Gui *et al.*, 2011; Shen *et al.*, 2012; Paolicchi *et al.*, 2013). Inactivating mutations in other KDMs such as KDM5A have also been linked to cancer, supporting the notion that these proteins can have a tumour suppressor function (Dalgliesh *et al.*, 2010).

Although presently there is much less information linking KDMs to other therapeutic areas, there is suggestive genetic evidence that altered KDM activity may be relevant to a number of diseases. For instance, single nucleotide polymorphisms (SNPs) in KDM5A and KDM1A have been linked to the autoimmune diseases ankylosing spondylitis and Grave's disease respectively (Newby *et al.*, 2010; Pointon *et al.*, 2011). KDM6B has also been implicated in an autoimmune disease based on its overexpression in antineutrophil cytoplasmic autoantibody-associated vasculitis (Ciavatta *et al.*, 2010). In addition, SNPs in KDM4C have been linked with autism, and SNPs in KDM3C have been linked with a number of metabolic and haematological parameters, while mutations in KDM5C cause a form of X-linked mental retardation (Jensen *et al.*, 2005; Yuan *et al.*, 2008; Chasman *et al.*, 2009; Soranzo *et al.*, 2009; Johnson *et al.*, 2010; Kantojarvi *et al.*, 2010). Finally, possible therapeutic applications in virus infections are suggested by the demonstrated role for KDM1A in alpha herpes virus reactivation from latency (Liang *et al.*, 2009).

A number of small molecules have been described that inhibit the demethylase activity of KDM1A, the first histone KDM identified. Early KDM inhibitors were generated based on the homology of KDM1A/B with MAOs, which also use FAD as a cofactor. Many of these inhibitors, such as trans-2-phenylcyclopropylamine (PCPA) and paraglyne, are non-specific and broadly inhibit MAOs (Metzger *et al.*, 2005; Lee *et al.*, 2006; Culhane *et al.*, 2010). Derivatives of these molecules that possess some selectivity for KDM1A over MAOs have been produced, such as OG-L002 (>30-fold selective for KDM1A) (Liang *et al.*, 2013) (Table 8). Peptide-based inhibitors (N-propargyl lysine-containing H3 peptides) with greater potency and selectivity than the MAO inhibitors have also been developed, but these possess poor cell permeability and hence are of limited use to investigate cellular activity (Szewczuk *et al.*, 2007; Yang *et al.*, 2007; Culhane *et al.*, 2010; Dancy *et al.*, 2012). Conversely, hybrid molecules between PCPA and lysine produced cell active inhibitors with significant selectivity for KDM1A over MAOs (Ueda *et al.*, 2009; Ogasawara *et al.*, 2011) (Table 8). Selective KDM1 inhibitors have also been developed based on the homology of this enzyme to polyamine oxidases and on the basis of structural features of the KDM1 active site (Huang *et al.*, 2007b; 2009b; Wang *et al.*, 2011).

In recent years, considerable progress has also been made in the development of inhibitors targeting the more recently

discovered Jmj KDMs. Based on the requirement of these enzymes for α-KG as a cofactor, α-KG analogues such as N-oxalylglycine (NOG) and α-hydroxyglutarate and their derivatives have been investigated and shown to act as low potency non-selective inhibitors of this target class (Cloos *et al.*, 2006; Rose *et al.*, 2008; 2010; Hamada *et al.*, 2010; Chowdhury *et al.*, 2011). More potent compounds that function through α-KG competition have also been identified based on small molecule screens (Rose *et al.*, 2008; King *et al.*, 2010; Chang *et al.*, 2011). In addition, compounds showing selectivity for Jmj KDMs over NOG have been identified (Hamada *et al.*, 2010).

While most of these ligand-based inhibitors are promiscuous KDM inhibitors, some compounds with more selective activities have been reported. For example, the plant growth regulator daminozide was shown to be an α-KG-competitive inhibitor that is much more potent against KDM2 and KDM7 family enzymes than other KDMs (Rose *et al.*, 2012); other compounds with a biased activity against KDM2 and KDM7 family enzymes have been developed based on the crystal structure of KDM7B (Suzuki *et al.*, 2013). High-throughput screening has been used to identify inhibitors of KDM4C and KDM5B (Hutchinson *et al.*, 2012; Sayegh *et al.*, 2013). In addition, inhibitors showing selectivity for KDM4 subfamily enzymes have been developed using a peptide-based approach, in which an α-KG analogue was linked to a small histone peptide bearing the target of these enzymes, H3(7–14)K9me3 (Woon *et al.*, 2012). Finally, structural knowledge of mode of binding of the H3K27me3 peptide to KDM6B was used to drive the development of the compound GSK-J1, which has selectivity for KDM6A/B over other tested KDMs (Kruidenier *et al.*, 2012) (Table 8).

Given that many of the KDM inhibitors developed so far, while improving are relatively non-selective, the therapeutic utility of targeting specific KDMs remains to be determined. However, inhibitors with some level of selectivity have shown potentially promising effects in early preclinical models. For example, certain KDM1A inhibitors have been shown to inhibit proliferation of cancer cells *in vitro* and to block herpes simplex virus lytic replication and reactivation from latency (Liang *et al.*, 2009; Wang *et al.*, 2011; Willmann *et al.*, 2012). In addition, a cell-penetrant prodrug version of GSK-J1 (GSK-J4) was recently shown to inhibit the production of pro-inflammatory cytokines by human macrophages, supporting the notion of targeting KDM6A/B for inflammatory diseases (Kruidenier *et al.*, 2012) consistent with initial evidence in mouse macrophages (De Santa *et al.*, 2007; 2009).

Arginine methyltransferases

In addition to lysine residues, histone arginines are also subject to methylation (Di Lorenzo and Bedford, 2011). Such methylation is favoured by the presence of glycine-arginine rich sequences (GAR motifs), although these are neither necessary nor sufficient. Methylated guanidine nitrogen atoms on peptidyl-arginine residue confer differential packaging, structural changes and altered protein interactions. Additional levels of intricacy result from differential dimeric processing of mono-methylated arginine (MMA) into either an asymmetrical- dimethylarginine (ADMA) or symmetrical-dimethylarginine (SDMA) residue.

To date, there are 11 proteins generally accepted as being members of the family of protein arginine methyltransferases (PRMTs), identified either on the basis of demonstrable methyltransferase activity, typically using SAM as the methyl donor, or homology to other family members (Table 9) (Wolf, 2009). However, a systematic survey of the human genome identified 44 putative PRMTs based on sequence homology at the active site, suggesting that this number could be much higher (Richon *et al.*, 2011). The 11 commonly accepted PRMTs have been classified into subgroups with different profiles (Yang and Bedford, 2013) (Table 9). All PRMTs can generate MMA, while type I PRMTs (PRMT1–4, 6 and 8) generate ADMA and type II PRMTs (PRMT5, 9) produce SDMA. PRMT7 appears to generate only MMA and has been

Table 9

Arginine methyltransferases

Symbol	Family	Synonyms and other symbols	Reported histone substrate	Uni-ProtKB/Swiss-Prot assession number
PRMT1	Type I	ANM1, HCP1, IR1B4, HRMT1L2	H4R3 H4R3	Q99873
PRMT2	Type I	MGC11137, HRMT1L1	H4	P55345
PRMT3	Type I	HRMT1L3		O60678
PRMT4	Type I	CARM1	H3R17, H3R26	Q86X55
PRMT5	Type II	JBP1, SKB1, IBP72, SKB1hs, HRMT1L5	H3R8, H4R3	O14744
PRMT6	Type I	FLJ10559, HRMT1L6	H3R2, H2AR29, H4R3, H2AR3	Q96LA8
PRMT7	Type II, III	FLJ10640, KIAA1933, [Myelin basic protein]-arginine N-methyltransferase	H4R3, H2AR3, H3R2	Q9NVM4
PRMT8	Type I	HRMT1L4	Unknown	Q9NR22
PRMT9	Type II	FBOX11, VIT1, UBR6, FLJ12673, MGC44383	Unknown	Q86XK2
PRMT10	Not classified	LOC90826, FLJ46629	Unknown	Q6P2P2
PRMT11	Not classified	FBX10, FBXO10, FLJ41992, MGC149840	Unknown	Q9UK96

termed as type III PRMT, although its capacity to produce SDMA remains a subject of debate (Zurita-Lopez *et al.*, 2012). The most recent family members (PRMT10-11) await definitive characterization. PRMT1 has been proposed to be the dominant cellular PRMT enzyme on account of its driving 85% of arginine methylation in diverse cells (Tang *et al.*, 2000).

Most PRMTs are ubiquitously expressed, although PRMT8 is reported to be selectively expressed in the CNS (Lee *et al.*, 2005; Kousaka *et al.*, 2009). Lethal mouse phenotypes have been observed after knockout of at least two family members (PRMT1 and PRMT4), suggesting that these proteins have non-redundant functions (Pawlak *et al.*, 2000; Yadav *et al.*, 2003).

The functional consequences of protein arginine methylation are ranging wide across most physiological processes, including growing evidence for a role in epigenetic regulation. In accordance with such a function, nuclear shuttling or localization has been demonstrated for some PRMTs [e.g. for PRMT1 and PRMT6 (Frankel *et al.*, 2002; Herrmann and Fackelmayer, 2009)]. Conversely, PRMT3 appears to be predominantly cytosolic and hence may not have a physiological role in histone methylation (Frankel and Clarke, 2000). Although understanding of histone arginine methylation has historically trailed that of lysine methylation, PRMTs have been identified as members of transcriptional complexes and can be recruited onto promoters by the action of transcription factors such as NF-κB and p53 (An *et al.*, 2004; Covic *et al.*, 2005). A growing number of PRMTs (including PRMT1, PRMT2, PRMT4, PRMT5, PRMT6 and PRMT7) are known to methylate different combinations of arginine residues on histones H2A, H3 and H4 (Table 9) with effects on chromatin accessibility.

Arginine methylation (and ADMA vs. SDMA methylation) may be stimulatory or inhibitory to transcription, depending on the histone context, the modifying enzyme and the degree of dimethylation. Co-integration with other histone code modifications has been reported. For example, PRMT1-mediated methylation of H4R3 facilitates subsequent acetylation (Wang *et al.*, 2001) while PRMT6-mediated methylation of H3R2 prevents H3K4 methylation by the MLL complex, effectively repressing transcriptional elongation (Hyllus *et al.*, 2007). Conversely, histone H3K18 acetylation primes the histone tail for asymmetric dimethylation at arginine 17 (H3R17me2a) by PRMT4 (Daujat *et al.*, 2002; An *et al.*, 2004), while H3K9ac blocks H3R8 symmetric dimethylation (H3R8me2s) by PRMT5 (Pal *et al.*, 2004).

These subtleties further emphasize the combinatorial complexity and likely exquisite selectivity of such epigenetic changes. However, histone methylation represents only part of the regulatory transcriptional potential of PRMTs, which also includes methylation and modulation of transcription factors such as CBP (Chevillard-Briet *et al.*, 2002) and Tat (Boulanger *et al.*, 2005), effects on RNA stability and splicing, and genomic reorganization via methylation of AT hooks of nuclear scaffold proteins such as HMGA proteins (Sgarra *et al.*, 2003; Edberg *et al.*, 2004). In keeping with their broad effects, PRMT1 and PRMT4 are considered to function as general transcription factors. Although understanding of how methylarginine marks are subsequently 'read' to activate transcription is currently limited, a recent study reported that PRMT4-mediated asymmetric dimethylation of H3R17 (a stimulatory modification) facilitates transcription elongation through recruitment of the PAF1 complex to activate oestrogen-receptor-dependent gene transcription, suggesting a possible model that other PRMTs may also use (Wu and Xu, 2012).

Abnormal PRMT expression or activity is increasingly being associated with a growing list of diseases. At present, the major link is between PRMTs and cancer. In particular, PRMT1 is considered key for transformation by the MLL complex (Cheung *et al.*, 2007). However, studies also support a potential role for therapeutic intervention in pulmonary and viral disorders (Boulanger *et al.*, 2005; Sun *et al.*, 2012; Zakrzewicz *et al.*, 2012) as well as spinal muscular atrophy (Brahms *et al.*, 2001).

Aided by increased understanding of catalytic mechanisms and knowledge of a number of PRMT crystal structures, several interesting tool molecules have been identified (Wigle and Copeland, 2013) (see Table 10 for examples). Since the discovery of the first PRMT family inhibitor truly selective for methyltransferase activity [AMI-1 (Cheng *et al.*, 2004)], screens have been run successfully and novel chemical equity has been disclosed, including the cellular inhibitor RM65 (Spannhoff *et al.*, 2007). In a flurry of recent published activity, for example (Bissinger *et al.*, 2011; Hart *et al.*, 2011), the rational design of C21, a chloroacetamidine-bearing histone H4 tail analogue that acts as an irreversible PRMT1 inhibitor (Obianyo *et al.*, 2011), has been included. Progress towards selectivity within the PRMT family, originally thought challenging due to the high sequence conservation, is also encouraging (Dillon *et al.*, 2012; Dowden *et al.*, 2012), because such selectivity may prove necessary to maximize therapeutic index. Additional exemplars include potent PRMT4 inhibitors from BMS (Huynh *et al.*, 2009 and Wan *et al.*, 2009) and a PRMT3-selective inhibitor that is also reported to be the first allosteric inhibitor of PRMTs or indeed of any reader, writer, eraser of methyl marks (Siarheyeva *et al.*, 2012). These promising early probes raise the hope that, with appropriate lead optimization, molecules with suitable pharmacokinetic and development properties for *in vivo* and clinical testing may be identified.

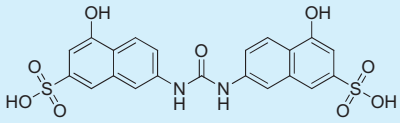
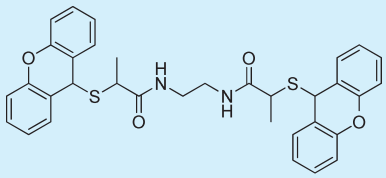
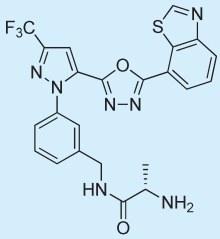
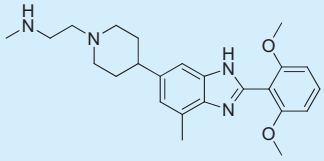
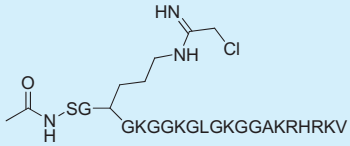
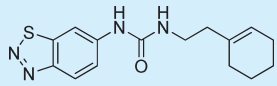
Arginine deiminases

Unlike the removal of lysine methyl marks, there is a scarcity of candidate enzymes with convincing demethylase activity against methylated arginines identified to date, with the exception of JmjD6 (Chang *et al.*, 2007). Consequently, it had been suggested that enzymes of the peptidyl-arginine deiminase (PAD) family, which are able to catalyse the deimination (or citrullination) of arginine side chains into citrulline moieties, could similarly act on methylated arginines, and reverse methylation in the process (Wang *et al.*, 2004). However, such catalysis is now thought to be unlikely under cellular conditions (reviewed by Thompson and Fast, 2006). Wider confirmation of the role of JmjD6 and the identification of additional demethylase enzymes therefore await further research.

Nevertheless, the PAD family remains a subject of interest as potential epigenetic regulators. The PADs include five

Table 10

Examples of PRMT inhibitors

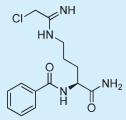
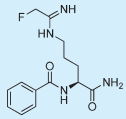
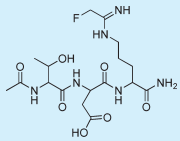
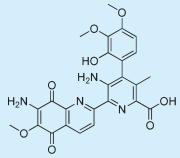
Inhibitor	Structure	Potency	Reported target	Reference
AMI-1		8.8 μM (PRMT1)	Pan-PRMT	(Cheng *et al.*, 2004)
RM65		55 μM	PRMT1 (not tested vs. other PRMTs)	(Spannhoff *et al.*, 2007)
BMS pyrazole inhibitor 7f		40 nM	PRMT4	(Huynh *et al.*, 2009)
Benzo[d]imidazole inhibitors of PRMT4 from BMS		70 nM (best exemplar)	PRMT4	(Wan *et al.*, 2009)
C21		1.8 μM	PRMT1 (PRMT6)	(Obianyo *et al.*, 2011)
Compound 1 (allosteric)		2.5 μM	PRMT3	(Jones *et al.*, 2012; Siarheyeva *et al.*, 2012) (Siarheyeva *et al.*, 2012)

members (PADs 1–4 and PAD6) with noted differences in function and substrate specificity (reviewed by Jones *et al.*, 2009). PAD4 has been the primary focus with regard to an epigenetic function, because it is the only family member with a clear nuclear localization sequence and it has been reported to citrullinate accessible arginine residues on the tails of various histones (most notably R2, R8, R17 and R26 on H3, and R3 on H4). However, in light of recent data suggesting that PAD2 can also be found in the nucleus (Jang *et al.*, 2011) and can effectively citrullinate histones (Zhang *et al.*, 2012), additional PADs may cause epigenetic modulation. PAD2 represents a potential target for multiple sclerosis based on its CNS expression and ability to citrullinate, and thereby destabilize, myelin basic protein (Oguz *et al.*, 2009). PADs 1 and 3 have roles in skin and hair follicle physiology, respectively, while PAD6 expression is limited to gametes and has not been shown to citrullinate any substrate to date.

Limited evidence for PAD4-mediated citrullination affecting transcription has come from a small number of reports, including effects on ER-responsive genes (Cuthbert *et al.*, 2004; Wang *et al.*, 2004; Zhang *et al.*, 2012) and p53-regulated promoters (Li *et al.*, 2008; 2010b). Interestingly, a reciprocal relationship between histone arginine methylation and citrullination has been demonstrated in a number of these studies [e.g. on p53 target promoters after UV treatment (Li *et al.*, 2008)], suggesting that citrullination may indeed be an indirect barrier to methylation via depletion of naive arginine residues, and that inhibitors of histone citrullination may have widespread transcriptional effects.

Citrullinated histones are also associated with the formation of neutrophil extracellular traps (NETs). These elusive structures (Brinkmann *et al.*, 2004; Yipp *et al.*, 2012) offer innate immunity functions through the trapping and killing of pathogens by extruded filaments containing DNA, histones and potent granule proteins. The initial discovery of citrullinated H3 and H4 epitopes (Neeli *et al.*, 2009; Wang *et al.*, 2009b) followed by the reported lack of NETosis and selective interference with host defence in PAD4-deficient

Table 11
Examples of PAD inhibitors

Inhibitor	Structure	Potency (μM)	PAD selectivity	Reference
Cl-amidine		5.9	Pan-PAD	(Luo *et al.*, 2006)
o-F-amidine		21.6	PAD4	(Knuckley *et al.*, 2010a; Causey *et al.*, 2011)
TDFA		2.3	PAD4	(Jones *et al.*, 2012)
Streptonigrin		1.87	PAD4	(Knuckley *et al.*, 2010b)

mice (Li *et al.*, 2010a) emphasizes that citrullination by this enzyme is a key feature in NETosis. There is increasing evidence supportive of the rationale that a selective PAD4 inhibitor may be effective in a wide range of diseases characterized by an excessive burden of NETs. These range from thrombosis (Martinod *et al.*, 2012) to systemic lupus erythematosus (Villanueva *et al.*, 2011), ulcerative colitis (Savchenko *et al.*, 2011), small-vessel vasculitis (Kessenbrock *et al.*, 2009) and sepsis (Clark *et al.*, 2007). NETs have also recently been linked to the pathogenesis of rheumatoid arthritis (Khandpur *et al.*, 2012), supporting historical non-epigenetic evidence for PAD4 being important in loss of tolerance to synovial proteins in this disease.

The first notable PAD inhibitors published were peptidomimetics, rationally designed to irreversibly modify a key active site cysteine residue via covalent attachment to a haloacetaminidine moiety (Luo *et al.*, 2006). The best studied exemplar, Cl-amidine, has subsequently been used widely as an *in vitro* tool. It has also demonstrated impressive efficacy in animal models of arthritis (Willis *et al.*, 2011), colitis (Chumanevich *et al.*, 2011) and lupus (Knight *et al.*, 2013), despite poor pharmacokinetics *in vivo* and an uncertain PK–PD relationship. Cl-amidine is also a non-selective inhibitor of all PAD enzymes and subsequent efforts have focussed on the development of second-generation inhibitors with increased potency and selectivity for individual PAD enzymes (e.g. see Table 11). Molecules such as o-F-amidine and Thr-Asp-F-amidine (Causey *et al.*, 2011; Jones *et al.*, 2012) demonstrate signs of increased selectivity for PAD4, while PAD3-selective probes have also been described (Knuckley *et al.*, 2010a). Encouraging signs of wider activity include the development of additional screens for PAD inhibitors and the identification of diverging and additional chemotypes (Wang *et al.*, 2012; Bozdag *et al.*, 2013). Ultimately, exploiting the binding determinants between the different PAD enzymes in order to identify and develop truly selective inhibitors for individual enzymes should allow definitive mechanistic understanding and guide optimal therapeutic positioning across a wider range of diseases.

Bromodomains

Acetylated lysines on histone proteins can be recognized by bromodomains (BRDs), which are small protein interaction modules of approximately 110 amino acids (Tamkun *et al.*, 1992). There are 61 human BRDs found within 42 different proteins (Table 12), with individual proteins containing between one and six BRDs (Figure 2). The three-dimensional structure of more than half of the family of BRD containing proteins (BCPs) has been experimentally determined (Figure 2), demonstrating a conserved hydrophobic pocket that accommodates acetyl-lysine side chains (Jacobson *et al.*, 2000; Nakamura *et al.*, 2007; Filippakopoulos and Knapp, 2012; Filippakopoulos *et al.*, 2012). BRDs are present in diverse nuclear proteins that possess intrinsic chromatin-modifying activity, including KATs (KAT2A, KAT2B, KAT4), KMTs (KMT2A, KMT2H), ATP-dependent chromatin-remodelling proteins (BAZ1B), helicases (SMARCA) and nuclear-scaffolding proteins (PB1) (Muller *et al.*, 2011). In addition, BCPs are often found as components of large protein complexes controlling chromatin architecture and recruit other proteins such as epigenetic writers and readers as well as transcriptional regulatory proteins to chromatin (Dawson *et al.*, 2011). Although the ability of BRDs to bind to acetylated lysine residues within histone proteins is linked to

Table 12

Bromodomain-containing proteins

Symbol	Synonyms and other symbols	Uni-ProtKB/Swiss-Prot assession number
ASH1L	ASH1, ASH1L1, huASH1, KMT2H, KIAA1420	Q9NR48
ATAD2	ANCCA, CT137, DKFZp667N1320, MGC29843, MGC5254, PRO2000	Q6PL18
ATAD2B	KIAA1240	Q9ULI0
BAZ1A	ACF1, hACF1, WALp1, WCRF180	Q9NRL2
BAZ1B	WSTF, WBSCR10, WBSCR9	Q9UIG0
BAZ2A	KIAA0314, TIP5, WALp3	Q9UIF9
BAZ2B	WALp4, KIAA1476	Q9UIF8
BPTF	FALZ, FAC1, NURF301	Q12830
BRD1	BRPF2, BRL	O95696
BRD2	KIAA9001, RING3, D6S113E, FSRG1, NAT	P25440
BRD3	KIAA0043, RING3L, ORFX	Q15059
BRD4	HUNK1, HUNKI, MCAP, CAP	O60885
BRD7	BP75, CELTIX1	Q9NPI1
BRD8	p120, SMAP, SMAP2	Q9H0E9
BRD9	FLJ13441	Q9H8M2
BRDT	BRD6, CT9	Q58F21
BRPF1	BR140, PEREGRIN	P55201
BRPF3	KIAA1286	Q9ULD4
BRWD1	C21orf107, WDR9	Q9NSI6
BRWD3	FLJ38568, MRX93	Q6RI45
CECR2	KIAA1740	Q9BXF3
CREBBP	CBP, KAT3A, RTS, RSTS	Q92793
EP300	KAT3B, p300	Q09472
KAT2A	GCN5, GCN5L2, HGCN5, PCAF-b	Q92830
KAT2B	PCAF, GCN5, GCN5L, P/CAF	Q92831
PBRM1	BAF180, PB1	Q86U86
PHIP	WDR11, BRWD2, DCAF14, FLJ20705, ndrp	Q8WWQ0
SMARCA2	BAF190B, BRM, hBRM, hSNF2a, SNF2, SNF2LA, Sth1p, SWI2, SNF2L2	P51531
SMARCA4	BAF190A, BRG1, FLJ39786, hSNF2b, SNF2B, SNF2L4	P51532
SP100	–	P23497
SP110	IFI41, IFI75	Q9HB58
SP140	LYSP100	Q13342
SP140L	–	Q9H930
TAF1	DYT3/TAF1, KAT4, NSCL2, TAFII250, BA2R, CCG1, CCGS, TAF2A	P21675
TAF1L	TAFII210	Q8IZX4
TRIM24	hTIF1, RNF82, Tif1a, TIF1A, TIF1, RNF82	O15164
TRIM28	KAP1, RNF96, TF1B, TIF1B	Q13263
TRIM33	FLJ11429, KIAA1113, PTC7, RFG7, TF1G, TIF1G, TIF1GAMMA, TIFGAMMA, KIAA1113	Q9UPN9
TRIM66	C11orf29, KIAA0298, TIF1D	O15016
ZMYND8	PRKCBP1, KIAA1125, RACK7	Q9ULU4
ZMYND11	BS69	Q15326

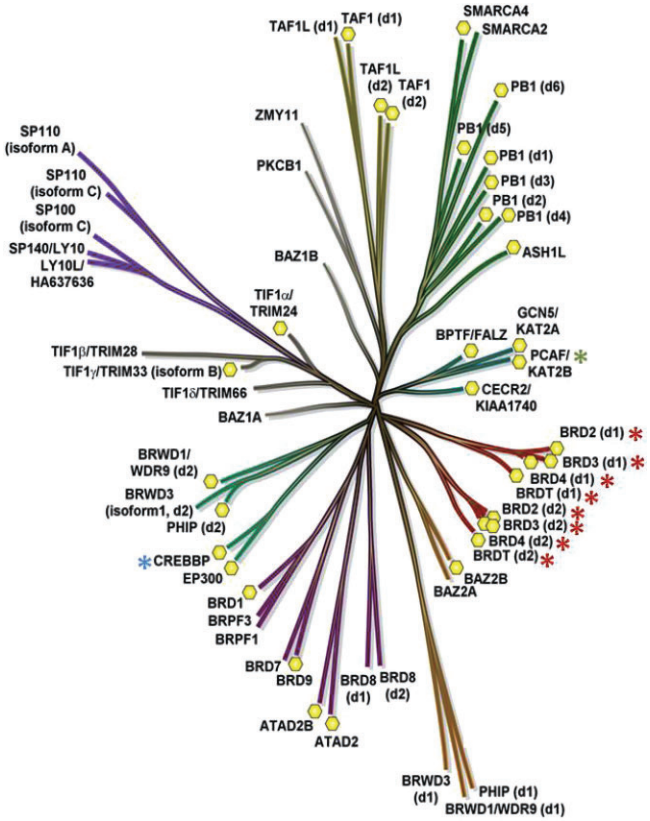


Figure 2

Phylogenetic tree of the human bromodomain family of proteins. The targets for which small molecule inhibitors have been identified are highlighted with asterisks. Yellow hexagons indicate X-ray structures in the public domain.

their gene regulatory activity, these domains have also been implicated in binding to non-histone acetylated proteins such as HIV Tat, RelA and p53 (Barlev *et al.*, 2001; Dorr *et al.*, 2002; Huang *et al.*, 2009a).

Recent studies have implicated BCPs in a wide range of human diseases, including cancer, inflammatory diseases, obesity, diabetes, infectious diseases, neurological disorders, and metabolic and cardiovascular indications (Taverna *et al.*, 2007; Prinjha *et al.*, 2012). Evidence for the role of BCPs includes altered expression in disease tissue, chromosomal translocations, amplifications and deletions involving BCP gene loci, genome-wide or focused gene sequence analyses linking SNPs to disease incidence, as well as phenotypes identified using knock-down or knockout studies. As an example, among the multiple BCPs reported to be overexpressed in tumours, which include ASH1L, BPTF, EP300, MLL, SMARCA2, SMARCA4, TRIM24 and TRIM28, ATAD2 has been shown to be up-regulated in various cancer types and to be significantly associated with prostate and endometrial cancer progression (Zou *et al.*, 2009; Raeder *et al.*, 2013), poor prognosis in breast and lung cancer (Caron *et al.*, 2010), and occurrence of metastasis and overall survival in breast cancer (Boussouar *et al.*, 2013). Furthermore, the identification of three sites of polymorphism in BRD2 associated with rheumatoid arthritis and the observation that Brd2-hypomorphic mice are severely obese and have reduced inflammation in fat tissue are examples of links of BCPs to inflammation (Muller *et al.*, 2011).

An understanding of the therapeutic relevance of the regulatory function of the BRD of BCPs is beginning to emerge with the recent development of small molecule BRD inhibitors (Table 13) (Chung, 2012; Hewings *et al.*, 2012). In some cases, these have been used to explore the interactions between BCPs and non-histone proteins. For example, compounds that bind to the BRD of PCAF with selectivity over the structurally related BRDs of CREBBP and TIF1β were identified by NMR-based small molecule screening and shown to disrupt the association of PCAF with HIV Tat-AcK50 *in vitro* (Zeng *et al.*, 2005). Likewise, ischaemin (Table 13), a selective modulator of the transcriptional co-activator CREBBP, is able to block the interaction of acetylated p53 (p53K382ac) with CREBBP, leading to regulation of tumour suppressor p53-induced transcriptional activity in cells and preventing apoptosis in ischaemic cardiomyocytes (Borah *et al.*, 2011).

The most advanced targets with respect to the development of BRD inhibitors are the members of the bromodomain and extraterminal (BET) subfamily of BCPs, which include BRD2, BRD3, BRD4 and BRDT (Table 13). Recently, a small number of potent, highly cell-permeable inhibitors with low nanomolar affinity for BET BRDs have been identified; these inhibitors appear highly selective for BET BRDs, but are active against the eight BRDs found in these four proteins due to their high degree of homology (Mirguet *et al.*, 2013). Among the diverse chemotypes reported to date are the first inhibitors disclosed, I-BET762 (GSK525762) and JQ1, both of which originated from chemical starting points found by phenotypic screening assays aimed to identify up-regulators of apolipoprotein A1 (Apo-A1), and I-BET151 and RVX-208 (Table 13).

BET inhibitors have shown promising effects in a variety of preclinical cancer studies. One cancer of particular interest is nuclear protein in testis (NUT) midline carcinoma (NMC), a rare, aggressively lethal tumour type in which chromosomal translocations between BRD4 (and sometimes BRD3) and the NUT protein play a causative role. JQ1 has been found to induce squamous differentiation and growth arrest in patient-derived BRD4-NUT-positive NMC cell lines and to decrease tumour size and improve survival in mouse xenograft models (Filippakopoulos *et al.*, 2010). In addition, BET inhibitors including I-BET762, I-BET151 and JQ1 have been shown to be active against myeloma (Delmore *et al.*, 2011), lymphoma (Emadali *et al.*, 2013), acute lymphoblastic leukaemia (Da Costa *et al.*, 2013), prostatic cancer (Gao *et al.*, 2013), neuroblastoma (Puissant *et al.*, 2013; Wyce *et al.*, 2013) and glioblastoma (Cheng *et al.*, 2013), *in vitro* and *in vivo*, while I-BET151 has been shown to have considerable preclinical activity against acute leukaemias, including MLL-fusion protein-driven leukaemia (Dawson *et al.*, 2011), and also against JAK2-driven myeloproliferative neoplasms (Wyspianska *et al.*, 2013). Similarly, I-BET726 was shown to induce cytotoxicity in mouse xenograft models of human neuroblastoma (Wyce *et al.*, 2013), and inhibition of BET has been shown to impair melanoma cell proliferation *in vitro* and tumour growth and metastatic behaviour *in vivo* (Segura *et al.*, 2013).

Table 13

Examples of bromodomain inhibitors

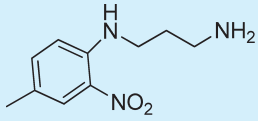
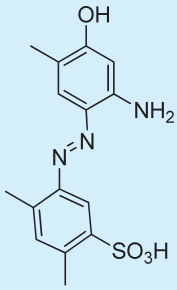
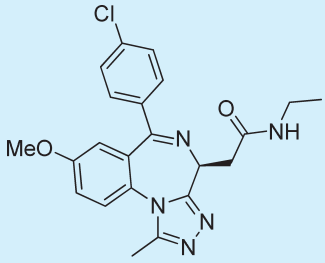
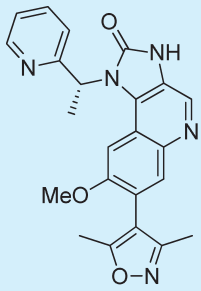
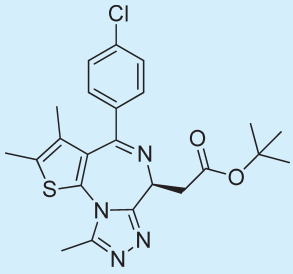
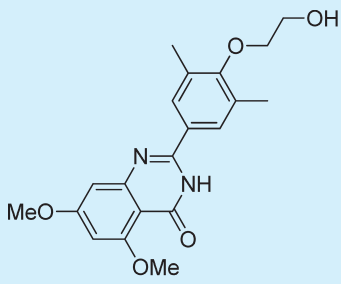
Inhibitor	Structure	Potency	Reported target	Reference
Compound 2		1.6 μM	KAT2B	(Zeng *et al.*, 2005)
Ischaemin (MS120)		19 μM	CREBBP	(Borah *et al.*, 2011)
I-BET-762 (GSK525762A)		50–60 nM	BRD2, BRD3, BRD4, BRDT	(Nicodeme *et al.*, 2010)
I-BET-151 (GSK1210151A)		250–800 nM	BRD2, BRD3, BRD4, BRDT	(Seal *et al.*, 2012)
JQ1		50–90 nM	BRD2, BRD3, BRD4, BRDT	(Filippakopoulos *et al.*, 2010)
RVX-208		40 nM – 3 μM	BRD2, BRD3, BRD4, BRDT	(Khmelnitsky *et al.*, 2013)

Table 14

Clinical studies of bromodomain inhibitors

Primary drugs	Sponsor	Trial phase	Disease type(s)	Trial ID
I-BET-762	GSK	I	NMC Leukaemias, lymphomas Multiple myeloma Myeloproliferative neoplasms	NCT01587703 NCT01943851
RVX-208	Resverlogix	I/II	Dyslipidemia Atherosclerosis Acute coronary syndrome Cardiovascular disease	NCT00768274 NCT01067820 NCT01058018 NCT01423188 NCT01863225
		II	Type 2 diabetes	NCT01728467
		II	Alzheimer's disease	(Study planned) http://www.resverlogix.com/programs/epigenetics/neurodegenerative-diseases
CPI-0610	Constellation Pharmaceuticals	I	Lymphomas	NCT01949883
		I	Leukaemias Multiple myeloma Myelodysplastic syndrome	(Study planned) http://www.constellationpharma.com/2012/09/constellation-pharmaceuticals-and-the-leukemia-lymphoma-society-partner-to-develop-novel-bet-inhibitor-for-the-treatment-of-hematologic-malignancies/
OTX-015	OncoEthix/ Mitsubishi Tanabe Pharma	I	Leukaemias, lymphomas Multiple myeloma Unspecified haematological cancer	NCT01713582

Based on these promising preclinical results, BET inhibitors are now entering clinical trials (Table 14). I-BET762 (Nicodeme *et al.*, 2010; Mirguet *et al.*, 2013), a benzodiazepine derivative developed by GlaxoSmithKline (GSK), was recently progressed into a phase I clinical trial for treatment of NMC, as well as other cancers (Mirguet *et al.*, 2013). In addition, CPI-0610 (Constellation Pharmaceuticals) and OTX-015 (OncoEthix) are also examples of BET inhibitors currently in phase I clinical trials for the treatment of various cancer types.

There is also great interest in the potential application of BET inhibitors in other therapeutic areas. In particular, there is considerable evidence that BET inhibitors may have utility in the treatment of autoimmune/inflammatory disease. In this regard, I-BET762 was shown to inhibit the expression of inflammatory cytokines and chemokines in activated mouse macrophages and to confer protection against LPS-induced endotoxic shock and bacteria-induced sepsis in mice (Nicodeme *et al.*, 2010). These anti-inflammatory effects were replicated by I-BET151, which reduced pro-inflammatory cytokine production by activated human peripheral blood mononuclear cells, and was effective at suppressing LPS-induced inflammation and sepsis in mice (Seal *et al.*, 2012), and also recently by JQ1 (Belkina *et al.*, 2013). Moreover, I-BET762 inhibited the ability of antigen-specific T-cells, differentiated under Th1 conditions *in vitro*, to induce pathogenesis in an adoptive transfer model of experimental autoimmune encephalomyelitis (EAE; Bandukwala *et al.*, 2012), and JQ1 was recently shown to suppress Th17 differentiation *in vivo* and to be protective in mouse models of autoimmunity (collagen-induced arthritis and EAE) (Mele *et al.*, 2013).

One of the most clinically advanced but significantly less potent BET BRD inhibitors is RVX-208 (RVX-000222) (Picaud *et al.*, 2013), which is currently being developed by Resverlogix Corp for the treatment of atherosclerosis and coronary artery disease based on its capacity to increase the levels of Apo-A1 and hence increase high-density lipoprotein cholesterol (McNeill, 2010) (Table 14). Toxicity and tolerability studies in animals and phase I/II clinical trials have indicated that RVX-208 is safe and well tolerated in multiple dosing regimens. It will be of great interest to see whether more potent BET inhibitors are similarly well tolerated in phase I trials mentioned above to gain an understanding of the therapeutic index of these inhibitors. RVX-208 is currently in phase 2 clinical trials for the treatment of atherosclerosis (Nicholls *et al.*, 2011). In addition, a phase I trial indicated that RVX-208 may have potential for the removal of β-amyloid plaques in Alzheimer's disease and this will be further assessed in an ongoing phase I/II clinical trial.

Like HDAC inhibitors, BET inhibitors have been shown to reactivate HIV from latency in cell lines and primary T-cell models, indicating their possible use in clearance and cure of the latent viral pool, as described above (Zhu *et al.*, 2012; Boehm *et al.*, 2013a,b; Li *et al.*, 2013). Their potential utility as therapeutics for heart failure has also been suggested recently based on the ability of JQ1 to block cardiomyocyte hypertrophy *in vitro*, and to prevent left ventricular hypertrophy and improve cardiac function in adult mice subjected to transverse aortic constriction (Spiltoir *et al.*, 2013). Finally, the efficacy of BET inhibitors in blocking the pro-fibrotic responses of idiopathic pulmonary fibrosis (IPF) lung fibroblasts and attenuating bleomycin-induced lung fibrosis in

mice provides a rationale to target the BET proteins for the treatment of IPF (Tang *et al.*, 2013b).

Concluding remarks

Although the basic scientific understanding of epigenetic regulatory mechanisms is at an early stage, great progress has already been made in taking drugs targeting these pathways into the clinic. The rapid pace of these developments has been driven by the recognition that dysregulation of epigenetic processes is apparent in diverse disease states, combined with the finding that many of the proteins that generate, remove and recognize histone modifications are tractable to small molecule drug development. In turn, the tractability of epigenetic targets has led to the generation of chemical probes that are being used to accelerate investigation into the biological function of these proteins.

As work in this young field continues, it will be of great interest to understand the full potential of this target class for the treatment of human disease. There will be many important questions relating to drug development for epigenetic pathways. For example, how many proteins within the multiple target classes of writers, erasers and readers will prove tractable to the generation of potent and selective small molecule modulators? Is there redundancy among these targets, and what role will polypharmacology play? What approaches can be taken to modulate epigenetic states in disease tissue while minimizing effects on normal cellular homeostasis? As well as acutely modifying gene expression, will it be possible to reset aberrant epigenetic states to normal, allowing for short-term treatments to induce remission or even 'cure' disease? Answering these questions will be aided by the continuing development of pharmacological tools together with increasingly sophisticated molecular approaches for elucidating the mechanisms that regulate chromatin structure and gene expression.

Author contributions

All authors contributed to writing of sections of the manuscript.

Conflict of interest

All authors are employees and shareholders of GlaxoSmithKline, which is researching and developing epigenetic inhibitors.

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