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Chromatin modifier enzymes, the histone code and cancer

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Abstract

In all organisms, cell proliferation is orchestrated by coordinated patterns of gene expression. Transcription results from the activity of the RNA polymerase machinery and depends on the ability of transcription activators and repressors to access chromatin at specific promoters. During the last decades, increasing evidence supports aberrant transcription regulation as contributing to the development of human cancers. In fact, transcription regulatory proteins are often identified in oncogenic chromosomal rearrangements and are overexpressed in a variety of malignancies. Most transcription regulators are large proteins, containing multiple structural and functional domains some with enzymatic activity. These activities modify the structure of the chromatin, occluding certain DNA regions and exposing others for interaction with the transcription machinery. Thus, chromatin modifiers represent an additional level of transcription regulation. In this review we focus on several families of transcription activators and repressors that catalyse histone post-translational modifications (acetylation, methylation, phosphorylation, ubiquitination and SUMOylation); and how these enzymatic activities might alter the correct cell proliferation program, leading to cancer.

Keywords: Histone code; Cancer; Histone acetylases; Histone deacetylases; Histone methylases; Chromatin

1. Introduction

In all eukaryotes, DNA is compacted into the nucleus as chromatin. The traditional view is that chromatin is required for packing the ~1 m length of the human genome DNA into the 10 μm diameter average size human nucleus. However, our view on the function of chromatin has become broader and more dynamic than just that of a DNA-packaging device. Chromatin represents an additional level of regulation for all DNA metabolic processes (replication, repair and gene expression) by working as a platform where biological signals integrate and molecular responses take place.

The structural subunit of chromatin is the nucleosome, which consists of 146 bp of DNA wrapped around an octamer of very basic proteins called histones. Each nucleosome core consists of two copies of each of the histones: H2A, H2B, H3 and H4 (Fig. 1). These evolutionarily conserved proteins have a globular C-terminal domain critical to nucleosome formation and a flexible N-terminal tail that protrudes from the nucleosome core. Nucleosomes assemble on an 11 nm filament array known as beads on a string, which undergoes a series of wrapping and compacting events as cells progress from interphase to metaphase, culminating with the totally condensed chromosome during metaphase.

Besides this generic organisation, local chromosomal domains present different levels of structure: heterochromatin was originally identified cytogenetically as the portion of the genome that remains condensed after the transition from metaphase to interphase. These regions correspond to telomeres and pericentric chromosomal areas and generally localise attached to the perinuclear compartment. Heterochromatic areas tend to be rich in repetitive sequences, low in gene content (although some genes are present), transcriptionally silent or showing a variegating phenotype and typically

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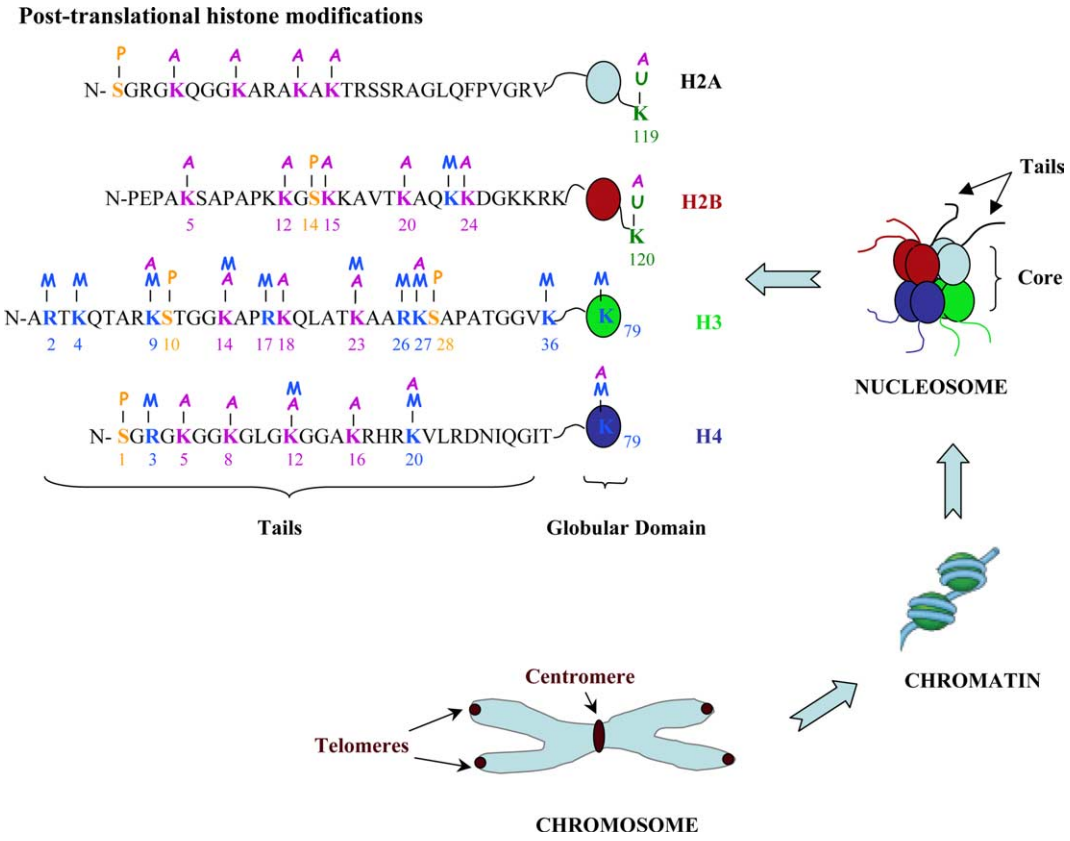

Fig. 1. Post-translational modifications on the histone tails. Modifications recently identified by mass spectroscopic techniques but unconfirmed (by mutational analysis and/or western blot with specific antibodies) are not shown. Note that Lysine 9, Lysine 14, Lysine 23 and Lysine 27 in the H3 tail and Lysine 12 and Lysine 20 in H4 can be either acetylated or methylated. Acetylation: purple; methylation: blue; phosphorylation: orange; ubiquitination: green.

replicate late. Euchromatin on the other hand may be considered as the rest of the genome, which de-condenses during interphase, contains most of the genes, is active or proficient for transcription and replicates early. In addition, chromatin organisation may change transiently in local areas of the genome as a response to cellular stimuli and/or differentiation programs.

The cell has developed mechanisms (below) to modify in a temporal/spatial manner the chromatin organisation and to ensure the maintenance of such an organisation through mitotic and meiotic cell division:

(a) ATP-dependent chromatin remodelling factors twist and slide nucleosomes, exposing or occluding local DNA areas to interactions with replication, DNA repair and transcription factors (reviewed in [1]).
(b) Post-translational covalent modifications of the histones within a nucleosome can either facilitate or hinder the association of DNA repair proteins and transcription factors with chromatin.
(c) Canonical histones in a nucleosome can be replaced by histone variants through a DNA-replication independent deposition mechanism. Histone variants harbour distinct information to respond to DNA damage conditions or to override an established gene expression stage (reviewed in [2]).
(d) Methylation at the C-5 position of cytosine residues present in CpG dinucleotides by DNA methyltrasferases (DNMTs) facilitates static long-term gene silencing and confers genome stability through repression of transposons and repetitive DNA elements. This is achieved through recognition of methyl-cytosine by specific methyl-DNA binding proteins that recruit transcriptional repressor complexes and histone modifying activities (reviewed in [3]).

The term "epigenetic" refers to the information contained in chromatin, other than the actual DNA sequence, that defines a heritable specific gene expression pattern. The above mechanisms, often operating in a coordinated way on a given locus, are responsible for the complex epigenetic network that controls gene expression programs in higher eukaryotes. Perturbation of epigenetic balances may lead to alterations in gene expression, ultimately resulting in cellular transformation and malignant outgrowth. In this review we will focus on the role of histone post-translational

modifications in the establishment and preservation of correct gene expression patterns and how deregulation and mis-targeting of these histone modifications contributes to the development of malignancies.

2. Histone post-translational modifications and "the histone code"

A variety of post-translational modifications occur on the amino terminal tail, as well as on residues located at exposed sites within the globular domain of the histones. These post-translational modifications include phosphorylation, acetylation, ubiquitination, methylation and SUMOylation (Fig. 1). Such modifications on histones can create or stabilise binding sites for regulatory proteins, like transcription factors, proteins involved in chromatin condensation or DNA repair. Histone modifications may also have the opposite effect, disrupting or occluding chromatin-binding sites. Accordingly, there are modifications that co-exist and work sequentially in a cooperative manner but are incompatible with others in the same nucleosome. That is the case for methylation of Lysine 4 H3 (K4 H3), acetylation of Lysine 14 H3 (K14 H3) and phosphorylation of Serine 10 H3 (S10 H3), all involved in transcription activation and incompatible with the generally inhibitory H3 Lysine 9 methylation (Fig. 2, H3).

Furthermore, the role of a particular modification in transcriptional signalling may also be influenced by the degree and stability of the modification. Lysine residues may be modified with one, two, or three methyl groups, and the "status" of histone methylation determines if transcription of certain genes is activated or repressed [4, 5; reviewed in 6].

Distinct histone modifications, on one or more tails, act sequentially or in combination to form a "histone code" that is read by proteins containing specific interacting domains: bromodomain and chromodomain. These proteins are the effectors that initiate downstream biological responses such as chromosome condensation, DNA repair or transcription activation/repression (reviewed in [7]). Examples of recruitment of chromo- and bromo-domain containing proteins, leading to different transcriptional read outs are shown in Fig. 3. Thus, although the basic composition of the nucleosome may be the same over long stretches of chromatin, the specific palette of modifications on nucleosomes creates local structural and functional diversity delimiting chromatin subdomains.

The molecular basis for how the epigenetic information carried in histone tail modifications is memorised

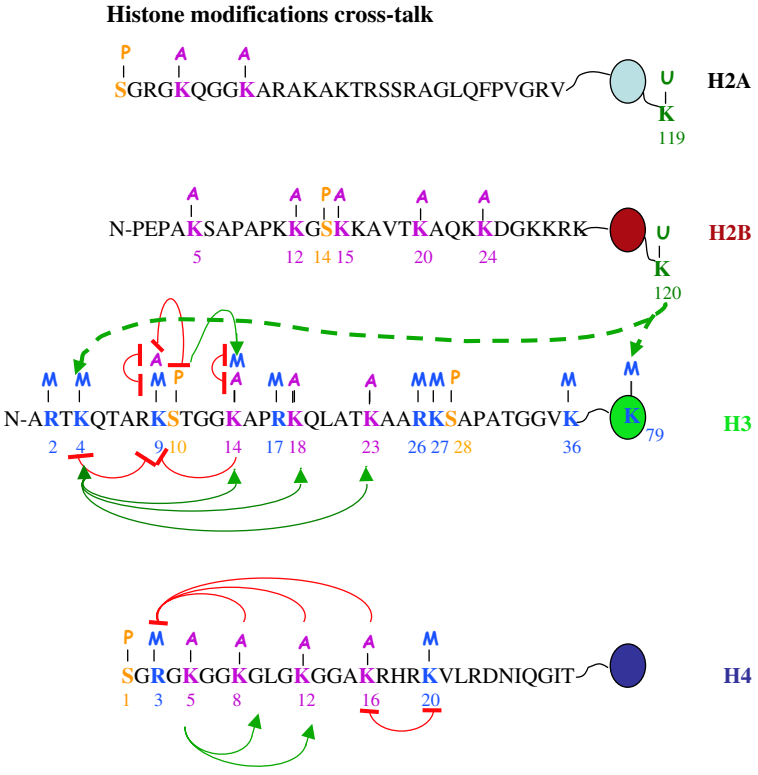

Fig. 2. Interplay between different post-translational modifications. "Compatible" modifications (those which facilitate other modifications to occur and/or can co-exist) are represented by green arrows. "Incompatible" modifications (those which negatively affect other modification and/or can not co-exist) are shown in red.

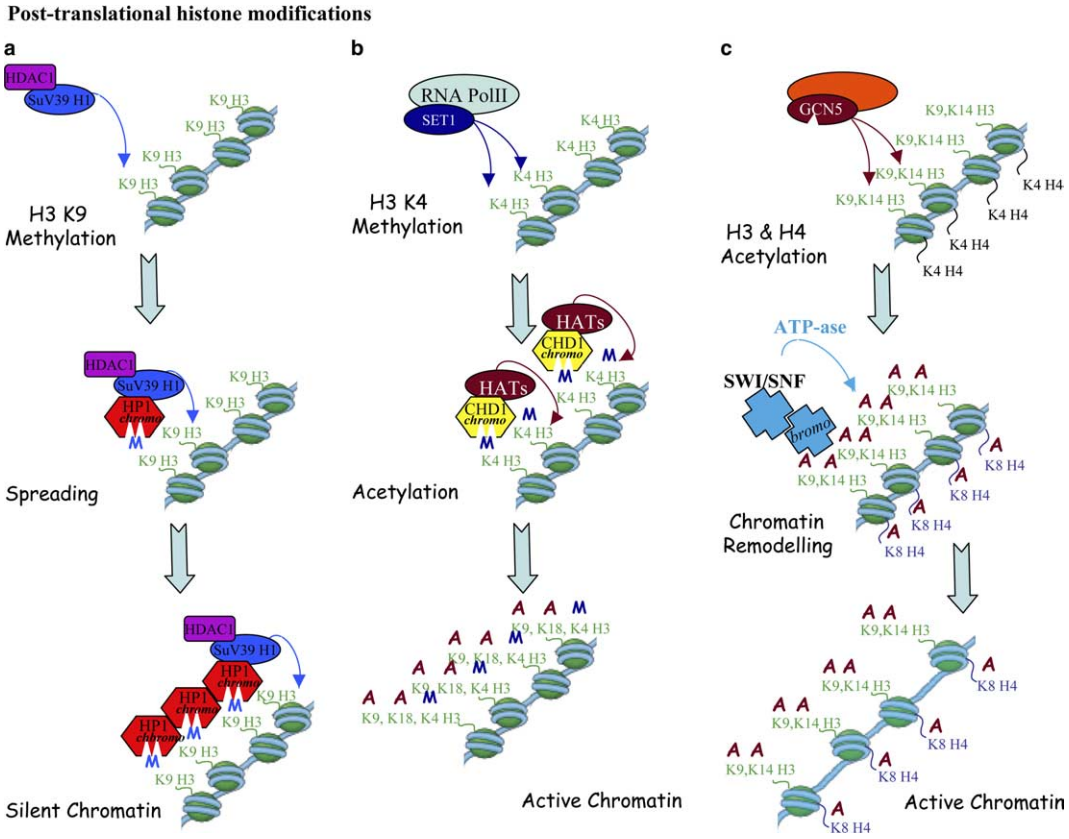

Fig. 3. Recruitment of bromo- and chromo-domain containing proteins by histone modifications. (a) Establishment of silent chromatin (heterochromatin) by lysine 9 H3 methylation: (1) SuV39H1 methylates lysine 9 H3. (2) Methylated Lysine 9 recruits the heterochromatin protein HP1 in physical association with SuV39H1. (3) Methylation of adjacent nucleosomes by SuV39H1 causes the spreading of the heterochromatin. (b) Establishment of transcriptionally active chromatin by lysine 4 H3 methylation: (1) Set1p methylates lysine 4 H3. (2) Methylated lysine 4 recruits the chromatin remodelling factor Chd1p in physical association with histone acetyltransferases. (3) Acetylation of lysine residues prevents repressive modifications to occur and recruits transcription activators. (c) Establishment of transcriptionally active chromatin by lysine acetylation: (1) GCN5 acetylates several residues within histones H3 and H4. (2) Acetylated lysines recruit the chromatin remodelling complex SWI/SNF. (3) SWI/SNF, via its ATPase activity, displaces and twists nucleosomes exposing DNA areas for interaction with the transcription machinery.

is unknown. Interestingly, biochemical data have suggested histones H3 and H4 are deposited into nascent nucleosomes as heterodimers [8]. This opens the possibility that the existing epigenetically coded H3/H4 dimers are divided on the two daughter strands, thereby forming the basis for an epigenetic memory imprint.

3. Histone acetylation and cancer

Acetylation of the e-amino group of lysine residues occurs on the four histones (Fig. 1). Broadly, acetylation of histones is linked to transcriptional activation. Therefore it is not surprising that many of the enzymes responsible for acetylation of histones at different residues where first known as transcriptional co-activators and later as enzymes. Most histone acetyltransferases take part in huge multiprotein complexes involved in locus targeting, thus providing chromosomal domain specificity in addition to the substrate specificity displayed by each individual acetyltransferase.

Based on sequence similarity histone acetyl transferases (HATs) can be organised into families, which seem to display different mechanisms of histone substrate binding and catalysis (Table 1).

The Gcn5/PCAF family of HAT proteins (GNATs) function as co-activators for a subset of transcriptional activators. They contain a HAT domain of around 160 residues and directly C-terminal to the HAT domain a conserved bromodomain, which has been shown to recognise and bind acetyl-lysine residues. The wide distribution of the bromodomain among enzymes that acetylate, methylate or remodel chromatin highlight the importance of lysine acetylation in self-maintenance of a transcriptional active state and recruitment of other sources of chromatin modifying enzymes (reviewed in [7]).

The p300/CBP family is another major group of nuclear HATs that has been extensively characterised (Table 1). The members of this family are more global regulators of transcription; contain a considerably larger HAT domain of about 500 residues, and other protein

Table 1
Human histone acetyl transferases (HATs)

Family	Substrate	Complex	Role
GNAT			
PCAF	H3/H4, TAT, E1A, p53, PCAF, AR	PCAF	T. Coactivator
GCN5L	H3/H4, TAFs	STAGA, TFTC	T. Coactivator
ELP3	H3/H4	Elongator	T. Elongation
P300/CBP			
P300	H2A/H2B/H3/H4, p53, EIA, TAT, AR		T. Coactivator
CBP	H2A/H2B/H3/H4, TFs, EIA		T. Coactivator
MYST			
Tip60	H3/H4/H2A, AR	TIP60	T. Activation
MOF (MYST1)	H3/H4/H2A	MAF2	T. Activation
MOZ (MYST3)	H3/H4		T. Activation
MORF (MYST4)	H3/H4		T. Activation
HBO1 (MYST2)	H3/H4		T. Corepressor; DNA replication
Transcription factors			
ATF2	H4/H2B		T. Activator
TAF1 (TAFII250)	H3/H4	TFIIB	T. Factor
TFIIIC90 (GTF3C4)	H3		T. Initiation
Nuclear hormone-related			
SRC-l (NCOAl)	H3/H4	NCOA	T. Coactivator
ACTR	H3/H4	PCAF/P300	T. Coactivator
Others			
CIITA (HMC2TA)	114		T. Coactivator
CDYL	H4		Protamine → histone
HAT1	H4/H2A		Histone deposition

GNAT: GCN5-related acetyltransferase; PCAF: EP300/CREBP-associated factor; TAT: tyrosine aminotransferase; AR: androgen receptor; TAFs: TATA box-associated factors; ACTR: activin receptor; CBP: CREB-binding protein; p300: e1a-binding protein p300; GCN5L: general control of amino-acid synthesis 5-like 2; GTF3C4: general transcription factor 3c, polypeptide 4; HBO1: histone acetyltransferase binding to ORC; MYST: MOZ, YBF2/SAS3, SAS2, TIP60 protein family; MOZ: monocytic leukemia zinc finger protein; MORF: MOZ-related factor; NCOA1/2: nuclear receptor coactivator 1 and 2; SRC, steroid receptor coactivators; HATl: histone aceayl transferase 1.

domains, including a bromodomain and three cysteine–histidine rich domains (*TAZ, PHD* and *ZZ*) that are believed to mediate protein–protein interaction.

The MYST family of HAT proteins are grouped together on the basis of their close sequence similarities, including a particular highly conserved 370 residue MYST domain, which uses an acetyl-cysteine intermediate in the acetylation reaction, so the catalytic mechanism involved is different from that shared by the other families of HATs (reviewed in [9]). The members of the MYST family are involved in a wide range of regulatory functions including transcriptional activation, transcriptional silencing, dosage compensation and cell cycle progression (Table 1). Besides the MYST domain, many members contain a cysteine-rich, zinc-binding domain within the HAT regions and N-terminal chromodomains.

As with bromodomains, chromodomains have been found in many other chromatin regulators, including remodelling factors and histone methyltransferases. Recently, it has been shown that the chromodomain of the heterochromatin protein 1 (HP1) and the yeast CHD1 protein (Chromo-ATPase/Helicase-DNA binding domain 1) can respectively recognise methylated K9 and K4 residues within the histone H3 tail [10–12]. Hence, it is not unreasonable to speculate that some chromodomain containing HATs might be recruited to chromatin by histone methylation.

Since the addition of an acetyl group to a lysine residue creates a new surface for protein association, and many transcription factors and chromatin regulators bind directly or indirectly acetylated histones, the maintenance of a specific histone acetylation pattern is crucial to cell proliferation. Consequently, it is not surprising that mutations or chromosomal translocations involving HAT genes result in development of malignancies (Table 4).

Several human histone acetyltransferases have been found to be involved in translocations where the resultant protein displays a 'gain-of-function' by deregulating HAT activity on histones or targeting lysine acetylation to new substrates. The p300 and CREB binding protein (CBP) genes are located on chromosomes 16p13 and 22q13, respectively, and are found rearranged in chromosomal translocations associated with leukaemia or treatment-related myelodysplastic syndrome. CBP fusion partners are the histone acetyltransferases Monocytic Leukaemia Zinc finger protein (MOZ) and MOZ related factor (MORF) [13]; and mixed lineage leukaemia (MLL), which encodes a K4 H3 methyltransferase (reviewed in [14]). The *MLL* gene, located at 11q23, is fused to the *p*300 and *CBP* genes

giving rise to MLL-p300 and MLL-CBP fusion proteins, in which the bromodomain, HAT domain and Q region of p300 or CBP are linked to the N-terminal part of MLL, which contains AT-hooks domains that can mediate targeting to DNA. The fusion of two HATs, MOZ and CBP/p300 creates a chimeric protein consisting of the N-terminal three-quarters of MOZ (including MYST and zinc finger domains, essential for HAT activity) fused to the C-terminal 90% of CBP, containing its HAT domain and a strong *trans*-activation region. The resultant protein harbours two active histone acetyltransferase domains, binding sites for several transcription activators and the H15 region of MOZ, which has been proposed to mediate interaction with nucleosomes [9]. Translocations involving MORF and CBP or p300 are also associated with acute myeloid leukaemia and with therapy-related myelodysplastic syndrome. The resulting fusion proteins are structurally similar to the *MOZ-CBP* and *MOZ-p300* fusion proteins described above. Albeit all analogies, developmental defects in MOZ zebrafish mutants suggest that MOZ and MORF are not just redundant cellular functions [15]. MOZ fusion with another transcription-related protein, TIF2, has also recently been reported in certain cases of leukaemia (reviewed in [16]). These translocations also contained an N-terminal portion of MOZ, fused to the C-terminal part of the nuclear receptor co-activator TIF2, including its putative CBP interaction and activation domains. Recently, it has been shown that *MOZ-TIF2* confers properties of self-renewal to committed myeloid progenitors *in vitro*, with the same domain requirement for both self-renewal and leukaemic transformation [17]. Finally, MOZ fusion with the steroid receptor co-activator 2, NCOA2, as a result of the pericentric inversion [8] (p11q13), has been reported in 6 cases of acute monoblastic leukaemia [18]. The high occurrence of HAT proteins among leukaemic translocations, highlight the importance of a tight balance of histone acetylation in the execution of the hematopoietic program. In fact, MOZ plays a role in HOX regulation in normal cells [15].

Besides translocations, mutations of some HATs are associated with cancer development. In this sense, histone acetyltransferases act as tumour suppressors. Consistent with this notion, mutations that inactivate alleles of p300 and CBP cause development of hematological malignancies in mice [19,20] and mutations in p300/CBP have been identified in several cases of human leukaemia [21]. Biallelic mutations of the p300 locus have been identified in human cancers of epithelial origin [22] and exogenous expression of p300 is able to suppress the growth of human carcinoma cells *in vitro* [23]. Monoallelic mutation of the CBP locus is the genetic basis for Rubinstein–Taybi syndrome (RTS), which has been reported to be associated with increased risk of developing malignant tumours. Several essential features of RTS are due to haploid insufficiency of the function of CBP, particularly the HAT [24,25]. Acetyltransferase activity targeted to non-histone substrates such as human or viral oncoproteins also contributes to the development of malignancies as will be discussed later.

4. Histone deacetylation and cancer

Histone lysine acetylation is a reversible post-translational process. The dynamic equilibrium of lysine acetylation *in vivo* is governed by the opposing actions of acetyltransferases and deacetylases. Deacetylation of histones by histone deacetylases (HDACs) results in a decrease in the space between the nucleosome and the DNA that is wrapped around it, thus, diminishes accessibility for transcription factors, modifying the chromatin from an open gene active euchromatin structure to a closed gene silenced heterochromatin structure. Similar to acetyltransferases, the HDACs are part of large multiprotein chromatin complexes, but in this case involved in transcriptional repression.

There are three major families of mammalian HDACs, based on homology to the yeast counterparts Rpd3, Hda1 and Sir2/Hst (Table 2). The class I HDACs, are nuclear proteins widely expressed in a variety of tissues. They show a high degree of structural homology and contain a zinc molecule at the active site as a critical component of their enzymatic pocket. This site is the main target for inhibition of deacetylase activity by most developed anti-cancer drugs. A zinc-active pocket also characterises the members of the Class II HDACs but, in comparison to class I, they have a narrower tissue distribution, are much bigger in size, and shuttle between nucleus and cytoplasm as part of their mode of action. Two members of this class, HDAC6 and HDAC10 are unique since they harbour two catalytic domains. The third HDAC family (Class III or SIR-HDACs) is quite different, both structurally and in the catalytic mechanism. Their enzymatic activity depends on the cofactor NAD^+, which breaks down during the histone deacetylation reaction resulting in *O*-acetyl-ADP-ribose and nicotinamide (NAM) (reviewed in [26]). Interestingly, NAM inhibits the catalytic activity of *SIR-HDACs* whereas it has been proposed that *O*-acetyl-ADP-ribose might function in a signalling pathway that couples SIR-HDACs activity with transcriptional silencing [27].

HDACs are found *in vivo* as part of multiprotein complexes with clear functions as transcription co-repressors (Table 2). There is more than one mechanism by which HDACs may function in cancer development. On one hand, an abnormal increase in HDAC activity may result in the transcriptional inactivation of tumour-suppressor genes like p53. In fact, HDAC4, 8, and 9 are expressed to

Table 2
Human histone deacetylases (HDACs)

Family	Substrate	Complex	Role
Class I			
HDAC1	Histones, TP53, E2F1	Sin3, NURD	T. Corepressor
HDAC2	Histones, YY1	Sin3, NURD	T. Corepressor
HDAC3	H4, RELA	NCOR1/NCOR2-GPS2 – TBL1X	T. Corepressor
HDAC8	Histones		T. Corepressor
Class II			
HDAC4	Histones	NCOR1/NCOR2	T. Corepressor
HDAC5	Histones		T. Corepressor
HDAC6	Histones		
HDAC7	Histones	Sin3, NCOR2	T. Corepressor
HDAC10	Histones	NCOR2	T. Repressor
Class III (Sir-tuins)			
SIRT1	p53		Cell proliferation
SIRT2	Histones, Tubulin		Cell cycle, cell motility

TP53: tumor protein 53, transcription factor; E2F1: E2F transcription factor 1:YY1:Ying-Yang 1, transcription factor; RELA: subunit of NF-κB.

Table 3
Human histone methyl transferases (HMTs)

Family	Substrate	Complex	Role
Arginine HMTs			
PRMT1 (HRMT1L2)	H4 (Arg3), ILE3, ETOILE, HNRPA2B1		T. Activation
PRMT4 (CARM1)	H3 (Arg1T, Arg26), TARP, CBP, PAB1	AR, PCAF, NCOA2, P300, NUMAC	T. Coactivator
PRMT5 (SKB1)	H2A, H4, SMN	Methylosome	Celi cycle, snRNP assembly
Lysine HMTs			
MLL1 (ALL-1)	H3 (Lys4)	SET1, MENIN	T. Activation, cell proliferation, Hematopoiesis
MLL4 (former MLL2)	H3 (Lys4)	SET1, MENIN	T. Activation
hSET1	H3 (Lys4)	SET1/ASH2/HCF1	T. Activation
SMYD3	H3 (Lys4)		T. Activation, cell proliferation
SET7/9	H3 (Lys4)		T. Activation, silencing
SET8 (PR-Set7)	H4 (Lys20)		Celi cycle, heterochromatin
DOT1L	H3 (Lys79)		T. Activation, silencing
SUV39H1/2	H3 (Lys9)	E2F1, E2F4	T. Repression, heterochromatin
Eu-HMTase1	H3 (Lys9)	E2F6	T. Repression
SETDB1 (ESET)	H3 (Lys9)		Hetechromatin, silencing
G9a (BAT8)	H3 (Lys9, Lys27)		T. Repression, silencing
EZH2	H3 (Lys9, Lys27)	EDD-EZH2	T. Repression, silencing

ILE3: subunit of NF-AT; ETOILE: HIV Rev activator; HNRPA2B1: HIV Rev trafficking; TARPP: Thymocyte cyclic AMP-regulated phosphoprotein; CBP: CREB binding protein; PAB1: poly (A)-binding protein; NCOA2: nuclear receptor coactivator 2; NUMAC: nucleosomal methylation activator complex; SMN: survival motor neuron.

a greater extent in tumour tissues than in normal tissues [28] and HDAC2 is overexpressed in tumours from mice lacking the adenomatosis polyposis coli (APC) tumour suppressor [29] (Table 5). On the other hand, the tumour suppressor RB requires the activity of Class I HDACs to exert its function (reviewed in [30]) and the tumour suppressor p53 represses the transcription of the DNA-repair helicase protein RECQ4 by a mechanism involving Class I HDAC activity [31]. Hence, mutations in this family of deacetylases may contribute to disease. Yet, the most common outcome of inhibition of HDAC activity is to trigger differentiation, growth arrest, and/or apoptosis of tumour cells *in vitro* and *in vivo*. These are the bases for the development of HDAC inhibitors as anti-cancer drugs (discussed below).

5. Histone methylation and demethylation and cancer

Methylation is another post-translational covalent modification that occurs on the side-chain nitrogen atoms of lysine and arginine on histones. The most heavily methylated histone is H3 followed by H4 (Fig. 1). Arginine can be either mono- or dimethylated, with the latter in symmetric or asymmetric configurations. Lysine can accept one, two or three methyl groups, resulting in mono-, di-, or trimethylated forms. The different stages of methylation on a given residue, confer different biological read outs to the modified residue [4,6], thus methylation has greater combinatorial potential with respect to other modifications. In contrast to acetylation, which correlates almost without exception with transcriptional

Table 4
Histone acetyltransferase mutations in cancer

Gene	Mutation/rearrangement	Canter type	Reference
PCAF	Missense mutations	Epithelial cancer	[137]
P300	Bi-allelic mutations + LOH	Gastric carcinoma	[138]
	Stop codon mutations	Colon and breast cancer	[22]
	Missense mutations	Colorectal, gastric and epithelial carcinomas	[22,137,140]
	MOZ/p300 gene fusion	Acute monocytic leukaemia	[141–143]
	MLL/p300 gene fusion	Acute myeloid leukaemia	[144]
	Homozygous deletion	SiHa cervical carcinoma	[145]
	Point mutations	Rubinstein–Taybi syndrome	[146]
CBP	Stop codon mutations	Epithelial cancer	[137]
	In-frame deletion	Epithelial cancer	[137]
	In-frame deletion	Lung cancer	[147]
	Homozygous deletion	Lung cancer	[147]
	Missense mutations	Lung cancer	[147]
	MYST4/CBP gene fusion	Acute myeloid leukaemia	[148]
	MOZ/CBP gene fusion	Acute myeloid leukaemia	[142,149–154]
	MOZ/CBP gene fusion	Acute myelomonocytic Ieukaemia	[155]
	MORF/CBP gene fusion	Myelodysplastic syndrome	[156,157]
	MORF/CBP gene fusion	Acute myeloid leukaemia	[158]
	Internal tandem duplication+LOH	Esophageal carcinoma	[159]
	Stop codon mutation	Colon cancer	[130]
	MLL/CBP gene fusion	Theraphy-related leukaemia	[160–163]
	Deletions	Rubinstein–Taybi syndrome	[146,164]
	Intragenic duplications	Rubinstein–Taybi syndrome	[146]
	Point mutations	Rubinstein–Taybi syndrome	[165]
MOZ	MOZ/TIF2 gene fusion	Acute myeloid leukaemia	[142,166–168]
	MOZt(2;8)(p23;pl 1) translocation	MDS	[169]
MORF	MORFt(10;17)(q22;q21) translacation	Uterine leiomyomata	[170]
NCOA1	PAX3/ NCOA1 gene fusion	Rhabdomyosarcoma	[171]

LOH: Loss of heterozygosity.

Table 5
Histone deacetylase changes in cancer

Gene	Mutation/rearrangement	Cancer type	Reference
HDAC1	Overexpression	Mormone refractory prostate cancer	[172]
	Overexpression	Gastric cancer	[173]

MDS: myelodysplastic syndrome.

activation, histone methylation can result in either transcription activation or repression, depending on the modified residue and the palette of other modifications decorating the histone simultaneously (Fig. 3).

5.1. Histone arginine methylation

Protein arginine methyltransferases (PRMTs) catalyse the transfer of methyl groups from *S*-adenosyl-L-methionine (SAM) to the guanidino nitrogens of arginine residues [32] (Table 3). PRMTs share a conserved catalytic core, but have little similarity outside the core domain, that is, the amino- and/or carboxyl-terminal regions, which likely determine the substrate specificity and the ability to form oligomers [33].

The type I histone methyltranferases catalyse asymmetric dimethylation of arginine. This family include PRMT1 and the CARM1 (Coactivator-Associated R-Methyltransferase 1)/PRMT4, which, respectively, methylate histone H4 Arginine 3 and histone H3 Arginine 2, Arginine 17 and Arginine 26 [34,35]. Other less characterised members of this family are PRMT2 and PRMT3, both homologues of PRMT1 and contain SH3 domain and C2H2 zinc-finger motifs respectively, which may determine its substrate specificity [36,37].

The type II PRMTs catalyse the formation of symmetric dimethylarginine. This group include PRMT5/JBP1 [38] and the novel PRMT7 [39]. PRMT5 associates *in vivo* with the ATPase-chromatin remodelling hSWI/SNF complex, and display substrate specificity for Arginine 8 (R8) H3 and R3 H4 as preferred sites of methylation. PRMT7 contains two binding sites for the donor *S*-adenosyl-L-methionine (SAM) and exhibit histone methyltransferase activity against H4 [40].

Histone arginine methylation correlates with transcription activation of a variety of genes. Several laboratories have shown functional synergy between arginine methylation and histone acetylation in transcription activation events [41–43]. In fact, the arginine methyltransferases CARM1/PRMT4 and PRMT1 physically associate with histone acetyltransferases to form co-activator complexes, which, acting in a cooperative manner, mediate the function of the transcription factor Nuclear factor κB (NF-kB) and the tumour suppressor p53 [44,45]. In contrast, PRMT5 has been identified in promoter complexes where it has been proposed to function as a transcriptional repressor by methylating histones [46,47].

Work from several laboratories argues for the importance of the arginine methyltransferases in the regulation of cell growth and proliferation: CARM1 is overexpressed in androgen-dependent and independent prostate carcinomas [48]. Overexpression of PRMT5 causes a reduction in the expression of the tumour suppressors *ST7* and *NM23* correlating with increased R8 H3 methylation and increased transformation properties of human cells [49]. Finally, the tumour suppressor DAL-1 (differentially expressed in adenocarcinoma of the lung)/4.1B, whose expression is lost in primary non-small-cell lung carcinomas, physically interacts with PRMT3 inhibiting the methyltransferase activity against its cellular substrates [50]. Thus, arginine methylation seems to be an important mechanism to regulate expression/function of genes involved in tumour suppression.

5.2. Histone lysine methylation

Lysine methylation occurs in several residues on histones H3 and H4. Some of these residues are also substrates for acetylation (Fig. 1). With almost no exception, the enzymes that catalyse the methylation of lysine residues of histones share strong homology in a 140 aminoacid catalytic domain known as the SET domain (standing for Su(var), Enhancer of Zeste and Trithorax) (Table 3). Lysine methylation is extremely diverse in its consequences as it can promote transcription activation, mediate transcriptional repression, trigger heterochromatin formation and even chromosome loss.

Methylation of K4 on histone H3 correlates with euchromatic areas proficient for transcription and trimethylation of K4 H3 specifically accumulates upon activation of transcription [4,5,51]. Methylated K4 creates a binding site for the chromodomain containing protein Chd1p, which recruits acetyltransferase activity to activate transcription [12]. K4 methylation also recruits chromatin-remodelling activity that contributes to the nucleosome changes necessary during transcription activation [52].

There are several human Lysine 4 H3 methyltransferases, which display homology to the yeast Set1 protein beyond the catalytic SET domain (Table 3). The enzymatic activity and possibly the substrate specificity of the ''Set1 family'' of enzymes depend on their assembly into a multiprotein complex, which is also evolutionarily conserved [53]. Some of the lysine 4 H3 methyltransferases are only proficient in catalysis of monomethylation (for example Set7/9) whereas others can catalyse up to tri-methylation (MLL1) due to the presence of specific residues in the catalytic domain.

Similarly to K4, methylation of K36 and K79 on histone H3 also ''marks'' euchromatic areas and correlates with transcription activation [54,55]. There are several human homologues of the yeast K36 methyltransferase Set2 protein (NDS1, NSD2/MMSET, WHSC1, NSD3, ASH1, HIF1) however, no catalytic activity has been reported so far for any of them. Still, all members are linked to transcription regulation and several of them are involved in human disease [56]. K79 methylation is catalysed by an evolutionary conserved protein called DOT1 [57]. This enzyme has a catalytic domain with a unique organisation that resembles more an arginine methyltransferase than a lysine methyltransferase. The fact that the substrate, K79, lies well inside the globular domain of the histone H3, whereas all other methylated lysines are in the exposed amino-terminal tail, may account for the structural peculiarities of these enzymes [58].

Methylation of Lysine 20 on histone H4 is catalysed by SET8 (Pr-Set7). This modification occurs at the onset of mitosis and is involved in chromatin condensation, necessary to ensure proper chromosome segregation [59]. The chromatin-associated factor HCF1 (herpes simplex virus Host-Cell Factor 1) regulates the cell cycle changes in K20 H4 methylation, inhibiting a PR-Set7-dependent switch during mitosis from monomethyl to dimethyl K20 H4 and preventing in this way defective mitotic chromosome behavior [60]. In contrast to humans, K20 H4 methylation in yeast does not have any impact in chromosome condensation and transcription but serves as a DNA damage signal to recruit checkpoint proteins to damaged DNA (discussed below).

Methylation of K9 on H3 (mainly tri-methylation) triggers formation of constitutive heterochromatin by serving as a mark to recruit the heterochromatin formation protein (HP1). K9 mono- and di-methyl forms are involved in retinoblastoma mediated transcriptional repression of euchromatic genes [61] and in establishment of facultative heterochromatin in the mammalian inactivated X chromosome [62–64]. Methylation of K27 on histone H3 is also a signal for transcription repression and maintenance of stable epigenetic silencing via recruitment of the Polycomb Repressive Complex (PRC1) [65–68]. Although K9 and K27 methylation might appear as redundant cellular functions, recent work demonstrates that both modifications exhibit distinct distributions at different

loci and overlapping but distinct patterns of Polycomb recruitment [69].

Methylation of K9 H3 is catalysed by the "SuV39 family" of histone methyltransferases, which include the proteins Suv39h1, Suv39h2, G9a, ESET/SETDB1 and EuHMTaseI. Although they have common substrate specificity, the catalytic activity of these enzymes differs: Suv39h1/Suv39h2 are tri-methylases whereas ESET/SETDB1 is a di-methylase, only proficient for tri-methylation in association with mouse ATFa-associated modulator (mAM) [70,71]. The G9a HMT is responsible for the vast majority of K9 H3 dimethylation and most monomethylation in mouse embryonic stem cells [72]. Methylation of K27 from histone H3 [73] is also catalysed by G9a, while similarly, EZH2, the catalytically active component of the Polycomb Repressive Complex 2 (PRC2), is capable of methylating K9 and K27 of histone H3 [65,66]. Surprisingly, EZH2 is structurally more similar to the K4 methyltransferases than to the SuV39 family.

In agreement with the biological functions of K4 and K27 methylation, their corresponding enzymes belong to the Trithorax Group (TrxG) and Polycomb Group (PcG) of proteins, positive and negative regulators of transcription respectively. A strict equilibrium between PcG and TrxG function is essential for the maintenance of heritable transcription patterns of the homeotic (*Hox*) genes during development, hematopoiesis, X-chromosome inactivation and control of cell proliferation (reviewed in [74]). Thus, it is not surprising that several lysine methyltransferases are closely related to human cancer (Table 6).

EZH2 is highly expressed in metastatic prostate cancer, lymphomas and breast cancer [75,76]. EZH2 transcription is tightly regulated by the RB/E2F pathway and ectopic expression of EZH2 is capable of providing a proliferative advantage to primary cells. Thus, EZH2 is a *bona fide* oncogene [77].

Chromosomal rearrangements involving MLL1 occur in more than 80% of cases of infant acute leukaemia and therapy-related leukaemia (reviewed in [78]). Most of these reorganisations involve the amino terminal part of MLL1, excluding the catalytic SET domain, fused to about 60 different partners. Thus, the classic model to explain the role of MLL1 in leukaemia was a gain of function for the fusion oncogenic protein that would target transcription activation or repressor activity to new substrates via the DNA interacting motif of MLL1 (reviewed in [79]). However, it is conceivable that alternative mechanisms might contribute to malignancy since this protein contains multiple functional domains (AT-hooks, PHD-fingers, transactivation domain and SET domain). In fact, dimerisation of MLL1 has been reported as a new mechanism for MLL1-dependent transformation [80]. Furthermore, recent work from several laboratories suggests a possible role for the enzymatic K4 H3 methyltransferase activity of MLL1 and other members of the "Set1 family" in tumourigenesis: (a) The MLL4 (former MLL2) complex binds to the tumour suppressor protein MENIN (Multiple Endocrine Neoplasia type I gene). This interaction is lost in tumour-derived cells, pointing to K4 methylation as the regulatory mechanism involved in the MENIN tumour suppressor function [53]. Moreover, MLL4 is amplified in epithelial cancers such as glioblastoma and pancreatic cancer [81]; (b) MLL1 is also found in physical association with MENIN [82]. Cooperative interaction between MENIN and MLL1 plays a central role in MENIN activity as a tumour suppressor since loss of function of either MLL or MENIN results in down-regulation of p27Kip1 and p18Ink4c expression and deregulated cell growth [83]; (c) SMYD3 is overexpressed in the majority of colorectal and hepatocellular carcinomas. Overexpression of the SMYD3 K4 H3 methyltransferase activity enhances cell proliferation [84]; and (d) HALR (MLL3) maps to chromosome 7q36, which is associated with leukaemia and developmental defects [85]. Thus, overexpression, mis-targeting and/or deregulation of K4 H3 methyltransferase activity might result in aberrant regulation of gene expression and cellular transformation.

5.3. Histone demethylation

Until very recently, the dogma was that methylation is an irreversible process. This conclusion was raised from the observation that the half-life of histones and methyl-lysine residues within them are the same (reviewed in [86]). In contrast to acetylation or phosphorylation, which have fast turn over and fit the expected features of a regulatory modification, methylation was favoured as a permanent mark and therefore associated more with defining chromosomal sub-domains (euchromatin *versus* heterochromatin) rather that specific transcriptional stages. However, this model could not account for the changes in H3 K4 and R17 methylation observed upon activation of transcription [4,87] or changes in K9 H3 methylation occurring upon repression of transcription within euchromatic areas [61].

The last half-year has witnessed the identification of the first arginine and lysine demethylases. As proposed by Kouzarides and co-workers, the removal of a methyl group from histones seems to be achieved in diverse ways (reviewed in [86]). Methylated arginines can be erased from histones by a deimination reaction that results in the conversion of arginine into citrulline. This process affects methylated and non-methylated arginines, thus, it is not properly an "arginine de-methylation event", although it is certainly responsible for the turn over of this modification. Deimination of arginines on histone H3 and H4 is catalysed by PADI4 [88,89]. *In*

Table 6
Histone methyltransferase mutations in cancer

Gene	Mutation/rearrangement	Cancer type	Reference
MLL1/ALL1	MLL-PTD	Acute myelogenous leukaemia	[174–183]
	MLL-PNTD	Acute lymphoblastic leukaemia	[184]
	MLL gene amplification	Acute myelogenous leukaemia	[185–196]
	MLL gene amplification	MDS	[185,186,191,195,197]
	MLL gene amplification	RAEB	[198]
	MLL gene amplification	Acute lymphoblastic leukaemia	[199]
	Trisomy 11	Acute myelogenous leukaemia	[176,200,201]
	Trisomy 11 + translocation	MSD + myelomonocytic leukaemia	[155]
	Trisomy 11 + PTD	Acute monocytic leukaemia	[161]
	Trisomy 11 + PTD	MDS	[202]
	Multiple rearrangements	Acute Leukaemia + MDS	[203–207]
	Multiple rearrangements	Erythroid leukaemia	[208]
	MLL/AF17 gene fusion	Acute myelocytic leukemia	[209–212]
	MLL/LASP1 gene fusion	Acute myelocytic leukemia	[213]
	MLL/LAF4 gene fusion	Acute lymphoblastic leukaemia	[214]
	MLL/AF10 gene fusion	Acute myeloid leukaemia	[215,216]
	MLL/AFIO gene fusion	Acute lymphoblastic leukaemia	[217]
	MLL/AF10 gene fusion	Acute monocytic leukaemia	[218]
	MLL/SEPTIN6 gene fusion	Acute myeloid leukaemia	[219,220]
	MLL/FBP17 gene fusion	Acute myeloid leukaemia	[221]
	MLL/Gephryn gene fusion	Acute monoblastic leukaemia	[222]
	MLL/AF15ql4 gene fusion	Acute myeloid leukaemia	[223]
	MLL/AF9 gene fusion	Acute myeloid leukaemia	[224–228]
	MLL/AF9 gene fusion	Acute lymphoblastic leukaemia	[229]
	MLL/CIP29 gene fusion	Acute myelomonocytic leukaemia	[230]
	MLL/RASGAP gene fusion	Acute myeloid leukaemia	[231]
	MLL/AF-lp gene fusion	Acute lymphoblastic leukaemia	[232]
	MLL/AF5Q31 gene fusion	Acute lymphoblastic leukaemia	[211,233]
	MLL/AF4 gene fusion	Burkitt-like lymphoma	[234]
	MLL/AF4 gene fusion	Acute lymphoblastic leukaemia	[235–243]
	MLL/ENL gene fusion	Acute lymphoblastic leukaemia	[244–246]
	MLL/ELL gene fusion	Acute myelomonocitic leukaemia	[247]
	MLL/ELL gene fusion	Acute myeloid leukaemia	[244]
	MLL/EEN gene fusion	Acute myeloid leukaemia	[248]
	MLL/GRAF gene fusion	Acute monocytic leukaemia	[249]
	MLL/LPP gene fusion	Acute myeloid leukaemia	[250]
	MLL/TET1 gene fusion	Acute myeloid leukaemia	[251]
	MLL/LCX gene fusion	Acute myeloid leukaemia	[220]
	MLL/AF6 gene fusion	Chronic eosinophilic leukemia	[252,253]
	MLL/AF3p21	Acute monocytic leukaemia	[254,255]
	MLL/NUP98 gene fusion	Acute myelocytic leukemia	[256]
	MLL/GAS7 gene fusion	Acute myeliod leukaemia	[257]
	MLL/AFX gene fusion	Acute leukaemia	[258]
	MLL/ACACA gene fusion	Acute leukaemia	[259]
	MLL/SELB gene fusion	Acute leukaemia	[259]
	MLL/SMAP1 gene fusion	Acute leukaemia	[259]
	MLL/TIRAP gene fusion	Acute leukaemia	[259]
MLL2	19ql3.1 amplification	Solid tumors	[81]
MLL3(HALR)	7q36 Deletion	Acute myeloid leukaemia	[260]
SMYD3	Overexpression	Colorectal/hepatocellular carcinomas	[84]
EZH2	EZH2 amplification	Primary breast tumor	[77]
	Aberrant expression	Hodgkin's lymphoma	[261,262]
	Overexpression	Breast carcinoma	[76,263]
	Overexpression	Prostate cancer	[75]

PTD: partial tandem duplication; PNTD: partial non-tandem duplication; MDS: myelodysplastic syndrome; RAEB: refractory anaemia with excess of blasts.

vivo, the estrogen-regulated *pS2* promoter undergoes deimination of H3 when the gene is downregulated, thus exhibiting opposing features to PRMT1 and CARM1 arginine methylation. Since replacement of arginine by citrulline avoids further methylation of this residue, re-setting has to take place either by the action of

aminotransferase enzymes that can convert citrulline back into arginine or replication dependent new histone deposition.

Histone lysine methylation can be reverted by an amino-oxidase reaction, which produces an unmethylated lysine. The first identified lysine demethylase, LSD1 (KIAA0601), is a FAD-dependent amine oxidase with substrate specificity for methylated K4 on histone H3 [90]. LSD1 is found in association with several transcriptional repressor complexes [91,92] and RNAi knockdown of LSD1 results in an increase K4 methylation and concomitant derepression of the target genes [90]. Surprisingly, LSD1 is also part of the transcription activation complex that contains the Lysine 4 H3 methyltransferase MLL1 [ALL-1] [93]. The presence of MLL1 and LSD1 in the same protein complex, suggests that a very fine balance between methylated and unmethylated Lysine 4 H3 is crucial for the control of target promoters.

The arginine and lysine de-methylases identified so far revert only specific methylation statuses: PADI4 acts only on unmethylated or monomethyl-arginine, while LSD1 can demethylate only di-methyl lysine. Hence, the discovery of demethylases does not conflict with the finding that certain methyl-statuses remain on promoters after transcriptional changes have been operated [94,95].

6. Other modifications

Phosphorylation is another covalent post-translational modification of histones. The main substrate for phosphorylation is histone H3. The amino terminal tail Serine 10 (S10) of H3 can be phosphorylated with completely opposite effects: S10 phosphorylation is necessary to initiate chromosome condensation in the pericentric heterochromatin, by recruiting condensin, and subsequent spreading throughout the genome during the G2–M phase transition in mitosis and meiosis (reviewed in [96]). Conversely, phosphorylation of S10 H3 has an important role in the transcriptional activation of eukaryotic genes by promoting acetylation of K14 on the same histone tail ([97]; reviewed in [96]). Mitosis-specific phosphorylation of histone H3 also occurs on S28 and Threonine 11 (T11) at the onset of chromosome condensation, suggesting that combination of mitotic phosphorylation at S10, S28 and T11 may have a different read out than the individual S10 phosphorylation coupled to transcriptional activation. Members of the aurora AIR2–Ipl1 kinase family catalyse histone H3 phosphorylation at S10 during mitosis in several organisms. Their activity is counterbalanced by type1 phosphatases (PP1) in a cell cycle regulated manner. Recently, it has been shown that the histone variant H2AX is phosphorylated at S139 by a member of the phosphatidylinositol 3-kinase-like kinase (PI3KK) family. This modification facilitates post-replication DNA repair by recruiting cohesin, a protein complex that holds sister chromatids together [98].

The ε-amino group of histone lysine residues are also subject to modification by ubiquitin and ubiquitin-like proteins such as SUMO (Fig. 1). Due to the large size of these modifications, it is not clear whether SUMOylation and ubiquitination directly affects nucleosomal structure or packing or whether this modification serves to promote/inhibit interaction with non-histone proteins, or both (reviewed in [99]). Histone ubiquitination is generally associated with increased gene expression. Actually, monoubiquitination of histone H2B in yeast is required for methylation of histone H3 at K4 and K79, two activating modifications [5,100–102]. The mechanism by which ubiquitination promotes methylation seem to involve recruitment of proteosomal ATPases by ubiquitin-modified H2B [103]. In contrast, modification of transcription factors and histones by SUMO is generally associated with decreased gene expression by improving the association of the substrates with HDAC1 and HP1, two transcriptional corepressors. Both SUMOylation and ubiquitination are reversible, and dynamic cycles of conjugation/deconjugation seem to be essential for the proper regulatory activity of these modifications [104,105].

7. Other substrates

In addition to catalysing histone acetylation, a number of HAT proteins, including CBP/p300 and PCAF, have been shown to acetylate a myriad of transcription-related proteins. These include DNA-binding transcription factors such as p53, ELKF, HMGI(Y), TCF, NF-kB, MyoD, GATA1, E2F1, HNF4, where acetylation has been shown to enhance the DNA-binding affinity of the affected protein; transcriptional co-regulators, like ATCR, b-Catenin, c-Myc and RB; and also general transcription factors, for instance TFIIE, TFIIF and TFIIB, which are known to be acetylated although the biological significance remains unclear.

Acetyltransferase activity targeted to non-histone substrates such as human or viral oncoproteins also contributes to the development of malignancies: the adenovirus E1A mediates its effects on cellular transformation by interacting with cell growth regulatory factors. E1A can be acetylated by CBP/p300 and by PCAF. Acetylation of E1A disrupts the association of E1A with transcription repressor complexes thus, promoting aberrant gene activation [106]. The c-MYC oncoprotein is a substrate of the acetyltransferases hGCN5/PCAF and TIP60. Acetylation of c-MYC by either mGCN5/PCAF or TIP60 results in a dramatic increase in protein stability. The data reported here suggest a conserved

mechanism by which acetyltransferases regulate c-MYC function by altering its rate of degradation [107].

SUMOylation and ubiquitination may affect transcription by modification of non-histone proteins including histone deacetylases (HDAC1), tumour suppressors (PML, p53) and transcription factors (c-myc and glucocorticoid receptor GR) (reviewed in [99]). Aberrant SUMOylation/ubiquitination results in defects in subcellular localisation and alters the degradation rate of factors important in regulating cell proliferation and differentiation. In this line, the PML-RAR fusion protein expressed in acute promyelocytic leukaemia, is not SUMO modified whereas arsenic trioxide, an effective treatment for this disorder, restores SUMOylation of the fusion protein [108].

Lysine methylation has emerged as a novel mechanism to regulate tumour suppressor p53. Methylation of one residue within the carboxyl-terminus regulatory region of p53 is catalysed by methyltransferase SET9/7 to stabilise the half-life of the protein and maintaining it restricted to the nucleus [109].

8. Working as a team: interplay between histone modification, DNA methylation, ATP-chromatin remodelling and small double-stranded RNA silencing

8.1. Cross-talk between histone modifications

The nature of the histone code predicts that histone modifications impinge on each other by acting as molecular switches, enabling or blocking the setting of other covalent marks (reviewed in [110]). It also predicts a chronology in the establishment of a specific modification pattern. Both assumptions seem to be true. It is known that in gene activation, phosphorylation of histone S10 H3 facilitates acetylation of K14 and methylation of K4, resulting in an open chromatin conformation [97,111]. S10 phosphorylation also facilitates acetylation of K9, thereby preventing the setting of repressive Lys 9 methylation marks [112]. Also on histone H3, K4 methylation facilitates acetylation by creating a specific binding site for the chromodomain containing protein Chd1, component of SAGA and SLIK HATs complexes [12]. Regarding timing, upon estrogen stimulation, H3 is acetylated initially at K18, then at K23, and finally methylated at R17 [43]. Also, ordered cooperative modification of histones seems to be essential for transcriptional activation by the tumour suppressor p53. In particular, methylation of R3 H4 by PRMT1 is followed by p300 acetylation of H4, which precedes the accumulation of CARM1 and consequent R17 H3 methylation [45].

The cross-talk can take place even between modifications on different histones. For example, ubiquitination of histone H2B K123 is required for an efficient methylation of K4 H3 and K79 H3, both involved in transcriptional activation [100–102].

8.2. Cross-talk with DNA methylation

DNA methylation is the most studied epigenetic mechanism. While the vast majority of the genome is unmethylated, promoters of certain number of genes undergo DNA methylation of CpG islands. This modification brings about an inheritable chromatin state of transcriptional repression. DNA methylation affects histone modifications and vice versa. Data in different systems suggest a range of models with respect to the temporal order in which this two epigenetic events occur and their impact on the process of transcription (reviewed in [113]). Methyl-CpG enriched regions target methyl-CpG-binding proteins, which in turn recruit repressor complexes containing histone deacetylases [114] as well as histone methyltransferases [115]. Thus, DNA methylation seems to precede histone modification in the establishment of heterochromatin. However, there are data supporting DNA methylation as a secondary event, induced by an already silenced chromatin. Studies on the kinetics of silencing of transgenes show that loss of histone acetylation and H3 Lys 4 methylation are the first steps in the sequence of events, leading to reversible transcriptional repression; methylation of K9 H3 and methylation of CpG sites on promoter DNA are later events that 'lock' the repressed gene in a stable silenced chromatin state [116]. Similarly, it has been shown in fungi that trimethylated K9 H3 marks chromatin regions for cytosine methylation [117–119].

The order of events leading to heterochromatin formation may differ from organism to organism and from gene to gene. In any case, the proper epigenetic control of gene expression requires the cooperation of DNA methylation and histone modifications, and disruption of either of those processes leads to aberrant gene expression seen in almost all human cancers.

8.3. Cross-talk with ATP-dependent chromatin remodelling

ATP-dependent nucleosome remodelling has been linked to histone modification either as a pre-requisite to facilitate accessibility of the modifying enzymes or as the read out of a certain modification.

Histone acetylation/deacetylation activities are coupled to ATPase chromatin remodelling: the human nucleosome remodelling activities CHD3 and CHD4 (chromo-ATPase/helicase-DNA binding domain 3 and 4) are components of the transcription repressor complex NRD [120], whereas the chromatin remodelling protein Chd1 (chromo-ATPase/helicase-DNA binding

domain 1) is a component of the SAGA and SLIK histone acetyltransferase complexes.

The K4 H3 methyltransferase MLL1 [ALL-1] physically interacts with the chromatin remodelling factor hSNF5/INI1 [121], which is a tumour suppressor. Furthermore, K4 H3 methylation serves as a mark to recruit ISWI to chromatin to activate transcription [122].

Finally, the chromatin remodelling complexes are in charge of the histone deposition that occur independent of DNA replication, which might set up the bases for the epigenetic inheritance of the histone modification patterns (reviewed in [123]).

8.4. Small double-stranded RNAs

There are two main RNA-guided epigenetic mechanisms: RNA-directed DNA methylation (RdDM), which results in covalent modification of cytosines in the DNA, and RNAi-mediated heterochromatin formation, which targets histone methylation (typically methylation of lysine 9 of histone H3) to centromeric areas.

The small RNAs, 21–26 nucleotides (nt) in length, produced by the RNaseIII enzyme Dicer, can direct epigenetic alterations, such as gene silencing and heterochromatin formation, by incorporating themselves into silencing-effector complexes and guide them to complementary homologous DNA sequences (reviewed in [124]). Although, so far most of the data regarding RNA-mediated epigenetic pathways come from insect, plants and fungi, increasing evidence supports the existence of such mechanisms in vertebrates. Mammals have counterparts of the RdDM enzymatic machinery and *de novo* methylation of cytosines outside of the CG dinucleotide context, which is the result of RdDM [125], has been reported in mammals [126]. The α-satellite repetitive array, present in all human centromeric regions produces transcripts, which are processed by Dicer [127]. Remarkably, in dicer-deficient chicken cells heterochromatic proteins (Cohesin and HP1) delocalise, indicating a disruption of heterochromatin targeting by the RNAi machinery.

The discovery of RNAi-mediated nuclear processes has increased the complexity of the epigenetic network: RNAi-mediated chromatin modifications are important to determine patterns of gene expression and chromosome behavior. Thus, the RNAi enzymatic machinery has the potential to contribute to diseases such as cancer and chromosomal disorders.

9. The histone code for DNA damage

As we have discussed, post-translational histone modifications set up a "code" that can be read by cellular factors bringing about specific responses. Although this review focuses on the regulation of gene expression, the histone code also transmits information to sense and respond to DNA damage [128]. Generation of double-strand breaks represents an important source of translocations and other gross chromosomal alterations frequently seen in cancer cells.

Phosphorylation of the histone variant H2AX (Serine 126) occurs extremely fast and propagates over ~100 Kb around a single double-strand break site. Phosphorylated H2A (γ-H2AX) contributes to repair by recruiting the sister chromatid cohesion factor, cohesin, which is important for efficient post-replicative double-strand break repair [98]. Recently, lysine methylation has been identified as a novel damage-specific histone mark: methylated K79 H3 contributes to DNA repair by targeting 53BP1 to DNA double-strand breaks [129] and methylation of K20 H4 controls recruitment of Crb2 to sites of DNA damage in yeast [130]. Hence, specific histone modifications seem to accumulate in "foci" at the damaged DNA sequences facilitating the recruitment of a subset of damage response proteins and contributing in this way to genome integrity.

10. Therapeutics and future perspectives

In contrast to genetic events, the possibility of reversing epigenetic codes may provide new targets for therapeutic intervention. DNA methylation is tightly connected to cancer development in two possible ways: on one hand, oncogenesis may result from hypermethylation of tumour-suppressor genes, whereas global genomic hypomethylation could enhance oncogene expression and genomic stability (reviewed in [131]). Genomic hypomethylation may also cause genomic instability since demethylation predisposes DNA to strand breakage and recombination within derepressed repetitive sequences (reviewed in [132]). Drugs that inhibit DMTs activity, such as procaine and zebularine, are on clinical trials as anti-cancer therapy (reviewed in [133]). Furthermore, the use of 5-azacytidine has already been approved by the US Food and Drug Administration for the treatment of myelodysplastic syndromes. Certainly, targeting epigenetic marks to control the progression of cancer is no longer science fiction.

Several cancer associated mutations and chromosomal translocations result in repression of transcription through abnormal recruitment or overexpression of HDACs. This is the rationale for the development of HDAC inhibitors as a new class of anti-cancer therapy. Currently, HDACs are molecular targets for the development of enzymatic inhibitors to treat human cancer, and six structurally distinct drug classes have been identified with *in vivo* bioavailability and intracellular capability to inhibit many of the known mammalian HDACs

(reviewed in [134]). Initial clinical trials indicate that HDAC inhibitors from several different structural classes are very well tolerated and exhibit clinical activity against a variety of human malignancies. Although the molecular basis for their anticancer selectivity remains obscure to date, the fact is that HDAC inhibitors have the potential to modulate additively or synergistically the activity of other therapeutic agents. Thus HDAC inhibitors, in combination with chemical drugs or radiotherapy, can reduce uncontrolled cell proliferation and apoptosis (reviewed in [134]).

Although treatment with DMTs and HDAC inhibitors results in overall positive effects in control of cell proliferation, they are not 100% selective as they often target all members of a family of enzymes rather than an individual one. In the past decade, the targeting and inhibition of specific mRNAs by RNA molecules has become the big challenge to achieve maximum specificity in anti-proliferative therapy (reviewed in [135]). Double stranded RNA (dsRNA) and small inhibitory RNA (siRNA), can selectively and efficiently inhibit expression of specific oncogenes, expressed in cancer cells but not in normal cells. Shutting down the expression of cancer-promoting genes by siRNA has proven to be an effective approach against several cancer models. Cells infected with viruses express long dsRNA that can trigger the induction of the anti-proliferative cytokines and interferons, thereby preventing spread of the virus. Taking advantage of this antiviral response, the dsRNA killing strategy (DKS), based on the *in situ* generation of dsRNA that can induce those antiviral defenses specifically in cancer cells, has been developed recently. DKS has the potential to be applicable to a wide range of tumours, emerging as a powerful tool for cancer treatment (reviewed in [135]).

The increasing evidence for a direct link between histone methyltransferases and cancer, together with the discovery of demethylases bring to focus these families of enzymes as putative targets for cancer therapy. SMYD3 is clearly involved in the development of colorectal and hepatocellular carcinomas, thus, it is an excellent therapeutic target [84]. SMYD3 is particularly attractive since it contains enzymatic activity and binds to a specific sequence of DNA. MLL1 and MLL4 are found in acute myelogenous leukaemia/myelodisplastic syndromes and solid tumours, respectively [78,79,81]. Future research needs to be done to firmly prove a role of overexpression of the enzymatic activity in development of malignancy as the first step for drug targeting. The identification and characterisation of novel histone demethylases is of great importance, in analogy to HDAC inhibitors therapy. Since some histone lysine methylations are marks for DNA damage, inhibitors of histone lysine demethylases could be useful in the control of genome reorganisations due to defects in DNA double-strand breaks.

In most cancers, the molecular network associated with malignancies is extremely complex, hence it is often necessary to target more than one gene. Combined therapies seem to achieve stronger and more selective responses, however, little is known about the interplay of different epigenetic mechanisms and the consequences in the global system of targeting one specific pathway. It is our challenge to understand the cross-talk of different epigenetic mechanisms in order to design, in the most rational way, new anti-cancer drugs. The recent description of loss of acetylation at K16 and trimethylation at K20 as a common hallmark of human cancer [136] raises the prospect of using cancer-specific histone modification 'signatures' for diagnosis and for targeted therapy.

Conflict of interest statement

None declared.

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