Cancers with wrong HATs: the impact of acetylation

Vincenzo Di Cerbo and Robert Schneider

Abstract

Lysine N-ε-acetylation is a post-translational modification that regulates the function of histone and non-histone proteins. In several malignancies, histone acetyltransferase (HAT) activities are disturbed as a consequence of various genetic or epigenetic alterations. In particular, HATs can function as tumor suppressors, helping cells control cellular proliferation and cell cycle, and also as oncogenes, because abnormal acetylation can activate malignant proteins and contribute to cancer. An impaired acetylation profile can be indicative of a pathological process, and thus evaluation of histone acetylation could be used as a predictive index of patient survival or therapy outcome. Therefore, epigenetic therapy might be a very effective strategy to defeat cancer. With the use of histone deacetylase inhibitors and acetylation modulators (e.g. HAT inhibitors, bromodomain inhibitors), we are paving the way for a future epigenetic drug control of human diseases.

Keywords: cancer; epigenetics; histone acetyltransferases; acetylation; histone modifications

EPIGENETICS IN CANCER

Epigenetics has changed the commonly accepted knowledge of cancer biology. As genetics was recognized as the major component responsible for the tumorigenic process in the past, today we tend to assign a pivotal role to epigenetics in triggering or supporting the cancer progression.

Many epigenetic players can cooperate to transform cells into cancer cells, as different classes of epigenetic factors have been found altered in cancers. Among them stand out histone modifiers [histone acetyltransferases (HATs), histone deacetylases (HDACs) and histone methyltransferases], chromatin remodelers, DNA modifiers (DNA methyl- and hydroxymethyltransferases) and noncoding RNAs [1–4], all of which have a direct or indirect effect on chromatin structure and dynamics.

Genetic factors mainly rely on loss- or gain-of-function of tumor suppressors or oncogenes, respectively, whereas epigenetic-driven tumorigenic alterations are based on (potentially reversible) alteration of enzymatic activities, giving rise to a more globally aberrant phenotype [5].

Typically, epigenetic alterations include aberrant DNA methylation and/or histone modification patterns, leading to altered gene expression of key regulator genes mainly involved in control of cell growth and proliferation as well as DNA repair or maintenance of genome stability [2, 6]. This suggests that restoring their expression to the physiological level, e.g. with the use of epigenetic modulators, may contribute to cancer resolution. Based on this, the use of epigenetic modulators as anticancer compounds has been proposed as a new therapeutic strategy for cancer and other diseases [7–9]. As a prime example, HDAC inhibitors (HDACi) have already been introduced in clinical treatments of cutaneous T-cell leukemia, and currently many more epigenetic modulators are undergoing screening process [10]. In fact, modulation of acetylation levels seems to be the

Corresponding author. Robert Schneider, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), CNRS UMR 7104/Inserm U964/Université de Strasbourg, 67400 Illkirch, France. Tel: +33 (0) 3 88 65 35 80; Fax: +33 (0) 3 88 65 0; E-mail: schneidr@igbmc.fr

Vincenzo Di Cerbo is a PhD student at Max-Planck Institute of Immunobiology and Epigenetics in Freiburg (Germany) and his research focus is chromatin dynamics and analysis of novel histone modifications.

Robert Schneider did his PhD at the LMU, Munich, and was a postdoc at the Gurdon Institute, Cambridge. He moved recently from the MPI of Immunobiology and Epigenetics, Freiburg, to the IGBMC, Strasbourg, where he leads the team 'Functional epigenetics and chromatin regulation'.

most straightforward and so far most successful way to restore the normal epigenetic pattern and ultimately the correct gene expression profile. This highlights the importance of acetylation in cancer biology.

In this review, we aim to explore the various levels of involvement of acetyltransferases in cancer and the general contribution of impaired acetylation patterns on tumorigenesis.

LYSINE ACETYLATION: UNLOCKING CHROMATIN FOR TRANSCRIPTION

Acetylation is a reversible post-translational modification that occurs on a wide range of proteins [11]. Although 80–90% of human proteins can be acetylated at their N-terminus, a process that is impaired in cancer [12], the most studied acetylation is N-ε-lysine acetylation. The enzymes responsible for this modification belong to the family of KATs [lysine (K) acetyltransferases], characterized by limited target specificity, as many can modify a broad range of proteins.

Histones are major substrates for acetyltransferases and HATs have been studied due to the strong link between this modification and gene regulation. The counterparts of these enzymes are the HDACs that catalyze the removal of the acetyl moiety. Based on catalytic mechanisms, HDACs can be divided into two groups: canonical HDACs are zinc-dependent enzymes, whereas sirtuins comprise an independent class of deacetylases that utilizes NAD^+ as a cofactor.

It has recently been demonstrated that both HATs and HDACs are required for gene expression and regulation [13, 14]. Neutralization of the positive charge of lysine by acetylation can potentially reduce the interaction of DNA with histones, thereby loosening up the chromatin structure and making the DNA more accessible to transcription factors [15]. Tail histone acetylation can also be 'read' by different protein factors through a bromodomain that specifically binds acetylated lysines [16]. Together, charge neutralization and recruitment of factors (e.g. transcription factors, remodelers, chromatin modifiers) encompass the main mechanisms of acetylation-mediated gene activation. Genome-wide studies have identified enrichment of acetylation of different lysines (both on histones H3 and H4) at regulatory regions of active genes (e.g. enhancers, promoters, transcriptional start sites), a pattern conserved from yeast to human [17–20]. In addition, levels of histone acetylation are often proportional to levels of gene expression [21]. However, this association is not strictly static; in fact acetylation must be set and removed in quite a fast manner [14]. Moreover, levels of acetylation must always be balanced in order to achieve a proper gene expression, and indeed both HATs and HDACs are required at the promoters of active genes to favor transcription [14].

It is evident that the impairment of the balance between acetylation and deacetylation can affect gene expression. This is in fact what happens in cancer cells with altered acetylation patterns. The underlying cause of these altered acetylation patterns can be a result of genetic lesions that use epigenetic mechanisms to carry out a pathological program. An altered acetylation profile can derive from three main scenarios: (i) abnormal recruitment of HDACs to the wrong loci (e.g. tumor suppressors); (ii) reduced activity of HATs (or potentially of deacetylases) due to e.g. haploinsufficiency or inactivating mutations, resulting in silencing of target genes (e.g. tumor suppressors) and/or altered regulation of non-histone substrates or (iii) increased HAT activity on the wrong targets (e.g. oncogenes), due to aberrant recruitment or overexpression (Figure 1). These mechanisms can alter the normal cell cycle, block or revert differentiation, impair apoptosis and facilitate proliferation. Hence, depending on the target genes, both hyperacetylation and hypoacetylation can contribute to the establishment and propagation of a cancer phenotype.

THE DOUBLE FACES OF HATS IN CANCER

HATs are divided into three families depending on their structural homology: Gcn5-related N-acetyltransferases (GNAT), MYST (acronym for the founding members MOZ, Ybf2, Sas2, TIP60) and orphan (p300/CBP and nuclear receptors) (Figure 2). To date, altered HATs have been reported in several types of cancers, particularly, epithelial and hematological malignancies [22, 23]. Novel high-throughput sequencing studies report an ever-increasing number of new cases, reinforcing the idea that HATs can play a central role in the physiopathology of cancers [24–26].

Several primary mutations such as amplifications, deletions, point mutations or translocations of HATs have been described (Table 1); however, in certain cases an altered expression profile (both up- and downregulation) of different HATs without
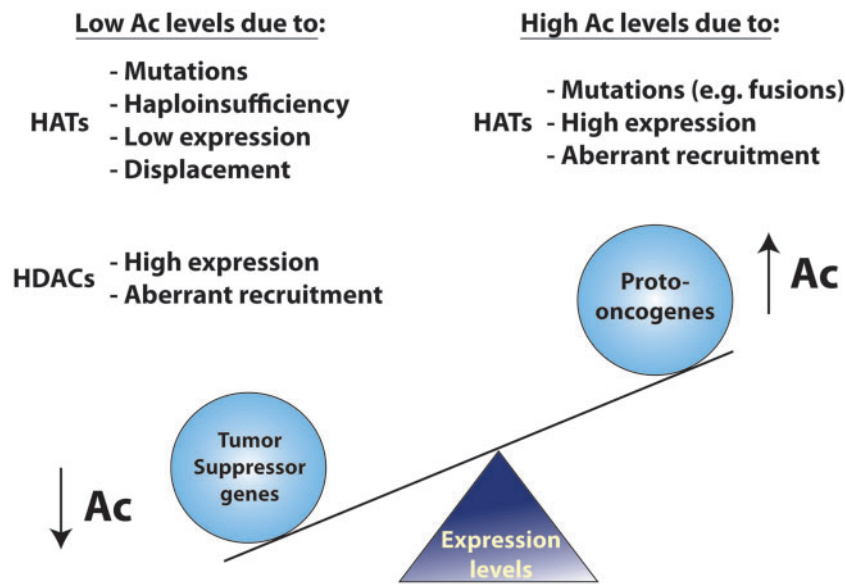
Figure 1: Acetylation imbalance can influence expression levels of tumor suppressors and proto-oncogenes. Several cellular alterations can lead to changes in acetylation on target genes in opposite ways. Hyperacetylation on proto-oncogenes can increase their expression and turn them into oncogenes, whereas hypoacetylation on tumor suppressor genes can silence them or reduce their expression levels. Therefore, an imbalance of the acetylation profile on different target genes can favor the tumorigenic process.

mutation has been reported [28–32]. This implies that the mechanisms implicated in malignancies involving acetyltransferases and regulation of acetylation seem to be rather diverse and affect a broad panel of factors, namely, various HAT substrates. Notably, considering the cellular functions of both HATs and their substrates, it emerges that these enzymes can act as either tumor suppressors or oncogenes, depending on the cellular or molecular context and cancer type (Figure 3).

HATS AS TUMOR SUPPRESSORS

Early studies on primary tumors and cancer cell lines identified somatic mutations in p300 and CREB-binding protein (CBP) genes associated with several types of cancers, including breast, colorectal and gastric cancers [33, 34]. Originally, these mutations seemed to be rather uncommon as only a small percentage of cases were identified. However, these studies established these two HATs as tumor suppressors and subsequently much proof-of-concept data confirmed this feature.

A typical tumor suppressor gene undergoes a phenomenon known as loss of heterozygosity (LOH), where a second 'hit' event can mutate the other 'healthy' allele, resulting in a biallelic null phenotype. Many cancer studies screened for LOH in cell lines and primary tumors to detect novel target genes and

identified mutations in p300 or CBP, although with very low frequency [35]. These mutations account for truncations, missense point mutations, deletions and insertions that involve the HAT or the cysteine–histidine-rich domains [34, 35]. Similarly, congenital heterozygous loss of one CBP allele results in the Rubinstein–Taybi syndrome, a multisystemic developmental disease characterized by mental retardation and physical defects, along with increased risk of childhood malignancies, with possible loss of the second allele [36].

More recent studies based on integrative genome sequencing in small-cell lung cancers [24] and non-Hodgkin B-cell lymphomas [25] have uncovered a higher frequency of alterations in these genes. These mutations are most often point mutations (less frequently fusions) in proximity to the HAT catalytic domain, resulting in loss of the enzymatic activity. Moreover, the heterozygous genotype determines a dosage of the gene product that is not sufficient to compensate for the required function (haploinsufficiency) and this could be one of the causes of the transforming phenotype. In acute lymphoid leukemia (ALL), a significant percentage of patients (18.3%) have mutations in the HAT domain of CBP, which impair its catalytic activity [26]. In these cases, the mutations were often associated with relapsed tumors, indicating that cells with a
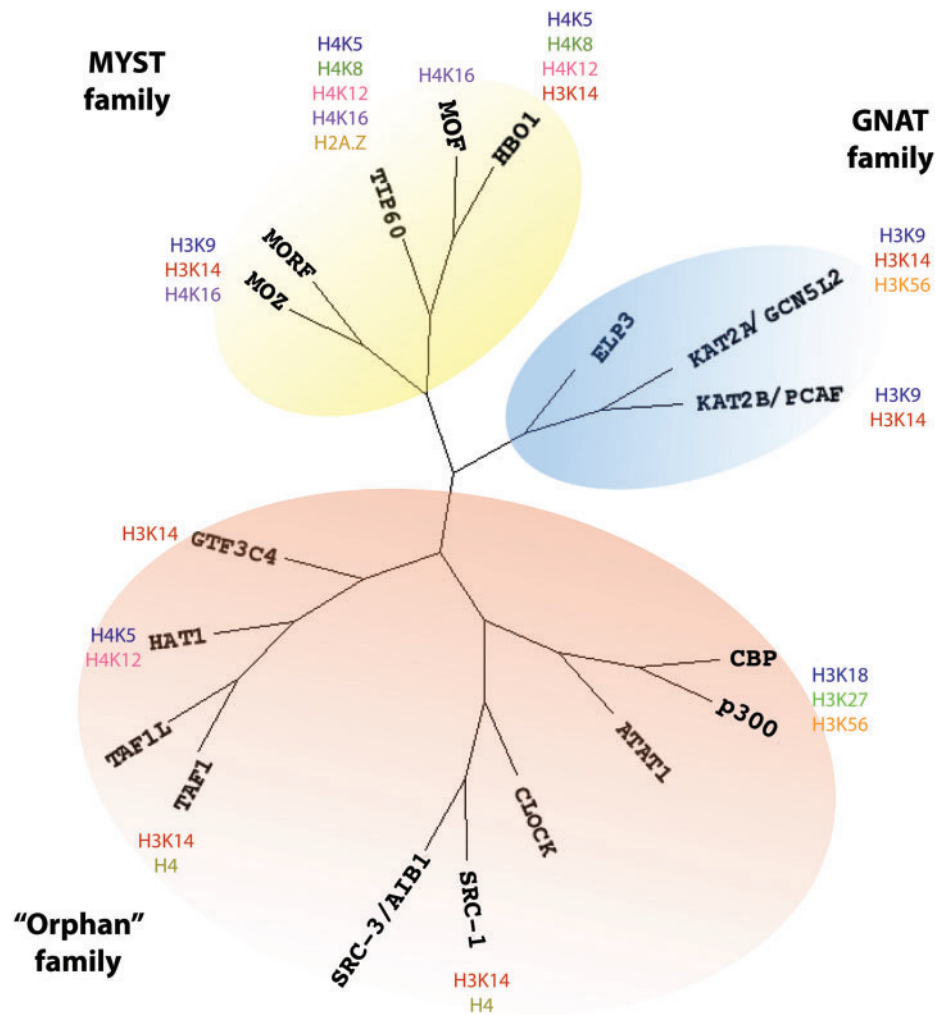
Figure 2: Phylogenetic tree of the HAT families. Members of each family are represented together with their major histone substrate. This refers to *in vivo* or *in vitro* data for HAT specificity reported in the literature. Note that many HATs have multiple targets and sometimes the literature is not clear. Adapted from http://www.thesgc.org [27].

mutated HAT can escape the first-line therapy. Most of these studies have focused on the identification of mutations in HATs; however, epigenetic mechanisms can also contribute to the phenotype, as in cases of LOH where the second allele is lost due to aberrant DNA methylation (hemizygosity) [37].

A further confirmation of the tumor suppressor nature of these genes derives from mouse experiments, where double null embryonic stem (ES) cells (−/−) for p300 or CBP were injected into blastocysts of different strains of mice. The resulting chimeras showed a significant incidence of hematological malignancies, all composed by cells lacking the HAT, demonstrating that they were causative of the tumor [35].

HATs are also able to modify non-histone proteins and the acetylation of different lysines on these

substrates has been shown to regulate their function. Among the most well-known substrates, there are p53 and pRb (retinoblastoma), key cell cycle regulators and tumor suppressors. Acetylation of p53, for example, is indispensable for its activation and stability, and the enzymes responsible for such modification are p300/CBP and TIP60/MOF [38]. It is then clear that disruption of HAT catalytic activity or even an altered dosage of the protein can affect stress response control mechanisms and promote tumorigenesis.

HIV Tat–interacting 60 kDa protein (TIP60) is one of the HATs involved in the regulation of apoptosis, DNA damage repair and Rb homeostasis, via its acetylation activity on many substrates beyond histones, such as p53, ataxia telangiectasia mutated and Rb [39]. It belongs to the MYST family of

Table 1: HAT mutations in cancer

Gene	Common name	Tumor types	Cell type	Tissue types	Mutation types	Fusion Proteins
MYST family						
KAT5	TIP60	Colorectal, head and neck, stomach	Somatic	Epithelial	Missense, frameshift, nonsense	
KAT7	HBO1	Lung, colorectal, breast, prostate, ovarian, sarcoma	Somatic	Epithelial	Amplification, missense, splice	
KAT6A/ MYST3	MOZ	Colorectal, lung, breast, acute myelogenous leukemia	Somatic	Epithelial, leukemia/ lymphoma	Nonsense, missense, amplification, deletion, translocation	MOZ-CBP, MOZ-p300, MOZ-TIF2, MOZ-NCOA3, MOZ-ASXL2
KAT6B/ MYST4	MORF	Colorectal, glioblastoma, lung, ovarian, acute mye-logenous leukemia	Somatic	Eepithelial, leukemia/ lymphoma	Nonsense, missense, amplification, dele-tion, translocation	MORF-CBP
MYST1	MOF	Lung, colorectal, medulloblastoma	Somatic	Epithelial	Missense, nonsense, deletion	
GNAT family						
KAT2A	GCN5	Breast, colorectal, prostate, lung, kidney, sarcoma	Somatic	Epithelial	Deletion, amplification	
KAT2B	PCAF	Lung, kidney, sarcoma, colorectal	Somatic	Epithelial	Missense, frameshift, deletion, amplification	
'Orphan' family						
EP300	p300	Colorectal, breast, pancre-atic, acute myelogenous leukemia, acute lympho-cytic leukemia, diffuse large B-cell lymphoma	Somatic	Epithelial, leukemia/ lymphoma	Translocation, non-sense, frameshift, missense, other	p300-MOZ, MLL-p300
CREBBP	CBP	Acute lymphocytic leukemia, acute myelogenous leukemia, diffuse large B-cell lymphoma, B-cell non-Hodgkin lymphoma	Somatic	Leukemia/ lymphoma	Translocation, non-sense, frameshift, missense, other	CBP-MOZ, CBP-MORF, MLL-CBP
CREBBP	CBP	Hematological (Rubstein−Taybe syndrome)	Germline	Leukemia/ lymphoma	Deletion	
NCOA1/ KAT13A	SRC1	Lung, colorectal	Somatic	Epithelial	Missense, deletion	PAX3-NCOA1
NCOA3/ KAT13B	SRC-3/ ACTR	Colorectal, ovarian, lung	Somatic	Epithelial	Nonsense, missense, amplification, in frame insertion	NCOA3-MOZ
KAT13D	CLOCK	Colorectal, glioblastoma, lung	Somatic	Epithelial	Missense, nonsense, amplification, other	
KAT4	TAF1	Lung, colorectal, breast, glioblastoma, ovarian, kidney	Somatic	Epithelial	Missense, nonsense, splice	

acetyltransferases and its locus (HTATIP) is fre-quently subject to mutation in head and neck squa-mous carcinomas, ductal breast carcinomas and low-grade B-cell lymphomas [40]. In these tumors, reduced mRNA and protein levels of TIP60 have been reported, accompanied by biallelic mutation of the locus at single nucleotide level (loss of heterozy-gosity) or gene silencing due to aberrant CpG methylation (hemizygosity), phenomena that seem to be prompted by p53 mutations [40]. It is then clear that the haploinsufficiency of TIP60 is driving the cell toward further mutational events,

contributing to cancer transformation. Reduction of TIP60 mRNA levels has also been reported in colon and lung cancers [41]. Taken together, we can define a role for TIP60 as a tumor suppressor gene. Similarly, male-absent on the first (MOF) also seems to play tumor suppressor functions, and its downregulation or mutation, associated with reduced H4K16ac, has been observed in medullo-blastomas and breast carcinomas [29].

Acetyltransferases are also key factors during viral infections. An interesting example of their contribu-tion comes from viruses coding for oncoproteins such
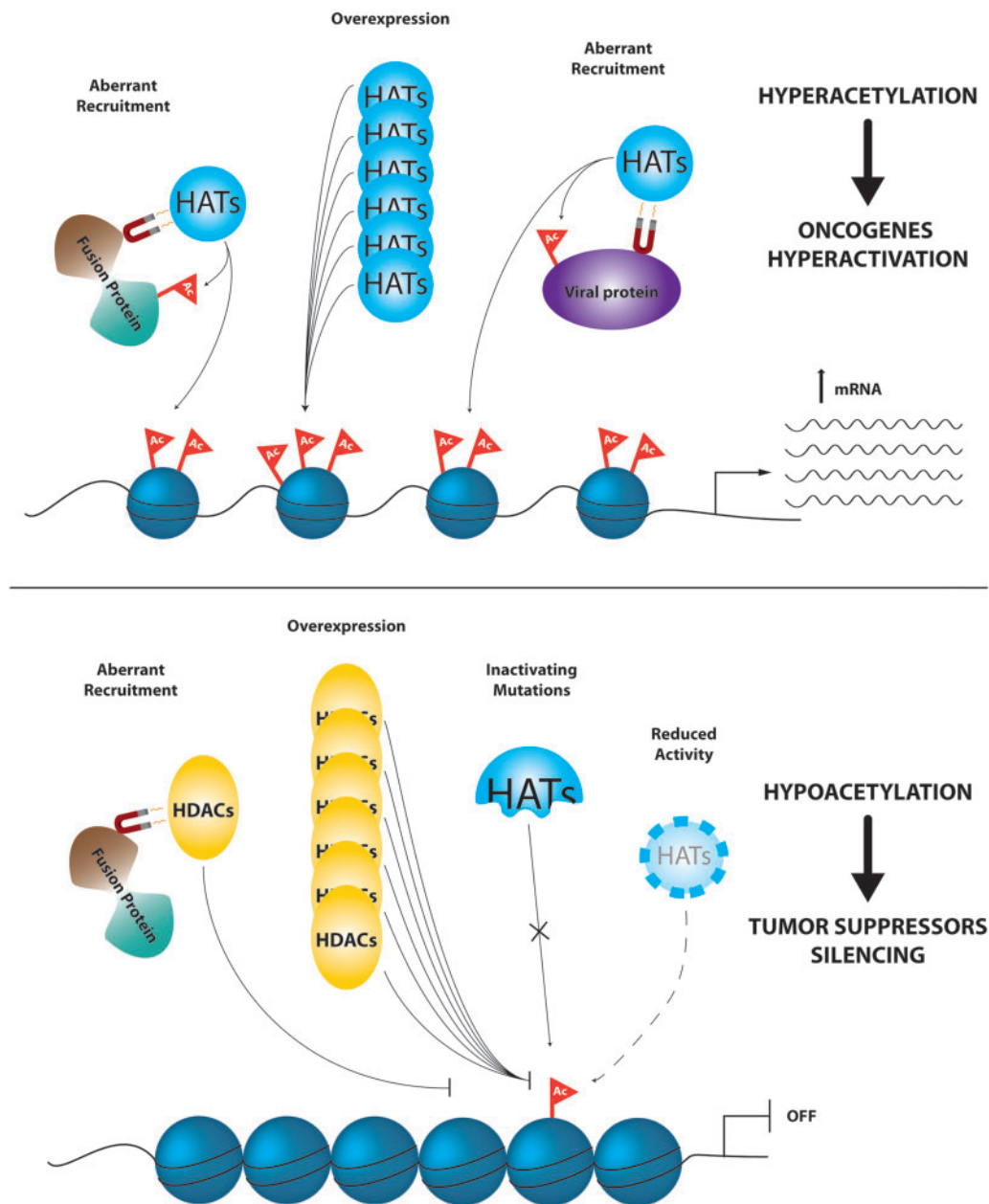
Figure 3: Mechanisms of oncogenic transformation involving histone acetyltransferases. Top panel represents the activity of HATs as oncogenes. Aberrant acetylation of histones at proto-oncogenes (middle) or acetylation of other tumorigenic players (e.g. oncofusion proteins, left, or viral oncoproteins, right) can hyper-activate these genes or proteins and boost carcinogenesis. Aberrant recruitment to the wrong loci or excess of the HATs due to pathological overexpression are typical mechanisms. Bottom panel represents possible ways in which a tumor suppressor gene can be silenced through hypoacetylation. Mutations in the HATs or reduced catalytic activity (e.g. for reduced expression or haploinsufficiency, right part) decrease the acetylation of tumor suppressors and hence gene expression. Similar effect is brought about by overexpression of HDACs or their aberrant recruitment by oncofusion proteins, left part.

as E1A (adenovirus), E6 (papilloma virus) or large T–antigen (SV40). These viruses are able to trigger cell transformation due to their viral oncoproteins that are able to interact with many cellular factors, including p300/CBP and Rb [35, 42, 43]. Cell

transformation is strictly dependent on this interaction. E1A is able to capture p300/CBP as well as p300/CBP-associated factor (PCAF), displace them from their regular genomic locations and target them to a novel subset of genes that are involved in cell growth,

division and DNA synthesis [42]. Simultaneously, genes depleted of p300/CBP and PCAF are involved in pathogen response and differentiation, and because they work as transcriptional coactivators, their loss induces gene repression [42]. In other words, this situation defines a dual function for these HATs. Indeed, the reduced availability of these enzymes prevents their activity as tumor suppressors, while their redistribution to specific genomic loci (proto-oncogenes) results in mistargeted acetyltransferase activity, conferring their oncogenic potential.

HATS AS ONCOGENES

When we consider acetyltransferases as cellular regulators, we also have to take into account the other side of the coin, namely, the hyper-activation of their catalytic activity that might be deleterious for the cell. The concept of the right dosage also applies to the excess of the enzyme, as an increase in their mRNA levels as well as misregulated activity can influence many cellular pathways and ultimately trigger or contribute to the cellular transformation into cancer.

A typical example of HATs primarily involved in cancer as oncogenes derives from a wide panel of hematological malignancies (e.g. ALL, acute myeloid leukemia (AML) or secondary AML), where despite the very low frequency of mutations in the HAT genes, the prognosis of patients is rather poor [44].

Chromosomal translocations are mutations that can result in novel fusion partners and generate chimeric proteins, usually endowed with novel oncogenic properties. Chromosomal breakage usually occurs at genomic hotspots, therefore only relatively few candidate genes take part to form these fusion proteins. In a few rare cases, primary translocations of HAT genes can generate chimeric proteins that retain HAT catalytic activity and bromodomains. This is the case for the mixed lineage leukemia (MLL)-CBP [t(11;16)(q23;p13)] or MLL-p300 [t(11;22)(q23;q13)] fusions, which account for 1% of the total MLL-fusions [45] and the MOZ-CBP [t(8;16)(p11;p13) or MOZ-p300 [t(8;22)(p11;q13)] fusions [46, 47], which are even rarer with about 0.4% of cases among AMLs [35]. Interestingly, these fusions can arise in patients that have been treated with topoisomerase II inhibitors for treatment of other cancers and consequently they can develop a secondary therapy-related leukemia (t-leukemia) [47].

Besides these cases where HATs are primarily involved in the oncogenic fusions, it has been

shown that p300/CBP are also able to modulate the activity of other more common fusion proteins. In particular, AML1-ETO [t(8;21)(q22;q22)], the most frequent fusion protein in AMLs, requires p300-mediated site-specific acetylation to induce leukemogenesis [48]. Notably, depletion of p300 via specific inhibitors or short hairpin RNA (shRNA) reduces the self-renewal potential of the transformed cells. In agreement with this observation, point mutation specifically of lysine 43 (K43), but not of a neighboring lysine also acetylated by p300 (K24R), into an unmodifiable arginine (K43R) significantly improves the survival of AML mouse models [48]. This clearly highlights the contribution of potent transcriptional coactivators such as p300/CBP to the cancer process and assigns oncogenic potential to this class of enzymes.

Similar mechanisms have been proposed for the fusion between monocytic leukemia zinc finger protein (MOZ) and the nuclear receptor coactivator TIF2 [inv(8)(p11q13)]. MOZ-TIF2 has lost the MYST catalytic domain, but nevertheless it requires the recruitment of CBP to function as an oncoprotein [49, 50]. Curiously, MOZ-CBP, which retains both HAT domains from each acetyltransferase partner, acts as dominant negative, as it blocks the AML1-dependent transcription program and inhibits macrophages differentiation [51]. This fusion cannot hyper-activate *per se* due to compromised functional activity (loss of MOZ transactivation domain at C-terminus). Instead, it might prevent the binding of the fully functional coactivators (i.e. wild-type MOZ and CBP) to the target genes and/or likely drive the acetylation activity toward aberrant histone and non-histone substrates [51].

The dosage effect seems to be a relevant feature of the regulatory mechanisms of HATs also in terms of overexpression. Indeed, in various cancers altered expression of HATs and other histone modifiers can be found [28]. Overexpression of p300 is observed in breast cancer[31], hepatocellular carcinoma [30], non-small lung cancer (NSLC) [32] and prostate cancer [52], all of which are associated with poor prognosis and survival. Prostate cancer is an interesting model to study the effect of oncogenic HATs. p300 as well as TIP60 [39] are able to acetylate the androgen receptor (AR) [52] even in a ligand-independent manner, therefore inducing expression of target genes. It has been shown that p300 particularly promotes prostate cancer progression via AR acetylation, which can be induced by the

interleukin-6 [52], and also via acetylation of NF-κB, which then becomes stabilized and can therefore potentiate the initial proliferative stimulus [53]. In resected prostate cancers treated with endocrine therapy (to reduce the AR–ligand availability), p300 and CBP expression is increased and the tumor can relapse. Inhibition of p300, but not of CBP, in prostate cancer cell lines with siRNA or HAT inhibitors (HATi) shows a significant decrease of the proliferation rate and induction of apoptosis [53]. This demonstrates that these HATs are able to sustain the therapy-resistant prostate cancer and their inhibition might contribute to tumor regression.

The ability of p300 to regulate steroid hormone receptors is also known for the estrogen receptors (ERs), whose aberrant stimulation is known to induce proliferation in breast and prostate cancers. When the tumor suppressor gene BRCA1 is mutated, p300/CBP acetylation activates ER-α in human breast and prostate cancer cell lines, whereas reintroduction of BRCA1 downregulates p300 but not CBP [54]. This mechanism might explain why p300 is upregulated in high-grade human breast cancers and is an indicator of poorer prognosis [31].

The effect of increased HAT activity can also be achieved without increasing the mRNA levels of the enzyme. For instance, increased production of polyamine, which is common in human skin cancers where the enzyme ornithine decarboxylase is upregulated, seems to enhance the protein levels and the HAT activity of TIP60 in mouse skin cancer models without affecting the mRNA levels. This results in increased histone H4 acetylation and an effect on transcription [55].

Altogether, these examples demonstrate that HATs can indeed play an oncogenic role in cancer cells. In fact, beyond malignancies in which HATs are primarily involved in the carcinogenesis, e.g. as oncofusion partner, there are many more cases where HATs are rather the epigenetic effectors of an oncoprotein, whose mechanism becomes thereafter supported by their acetyltransferase activity.

HISTONE ACETYLATION AS DISEASE BIOMARKER

Dysfunction of HATs is obviously reflected on the pattern of histone modifications. An accurate screening of the changes of these marks could reveal the status of the tissue and help predict the clinical outcome of a tumor.

Pioneering studies on changes in histone modification patterns upon cancer transformation have identified reduction in lysine 16 acetylation on histone H4 (H4K16ac) as a general hallmark of cancer cells concomitantly with loss in H4K20me3 [56].

With the advent of new high-throughput techniques such as tissue microarray analysis, which utilizes immunohistochemistry to analyze multiple samples from many patients simultaneously, it has become possible to evaluate changes in different modifications and expression of key factors and correlate them with clinical parameters (e.g. survival rate, grade of the tumor, progression-free disease). The first groundbreaking study made by Seligson et al. in 2005 demonstrated that combinations of various modifications, in particular H3K18ac and H3K4me2, could represent a reliable prognostic biomarker to predict the risk of tumor recurrence in chirurgical-treated prostate cancer patients. This study revealed that higher levels of these marks correlate with better prognosis and disease-free survival [57]. Subsequently, others have proposed a general prognostic rule for a diverse panel of histone marks in different cancers, including breast, pancreatic, gastric, kidney and lung cancer [57–61]. Generally speaking, it seems that a combination of changes in histone modifications can be predictive of patients' survival rate. Global loss of histone marks is often associated with a poorer prognosis [62]. In particular, loss of H3K18ac, H3K9ac and H4K16ac, or global hypoacetylation is associated with a shorter life expectation for postsurgical patients. Importantly, also loss in methylation marks such as H3K4me2, H3K9me2 and H3K27me3 [57, 58, 63] can also be used as biomarkers for poor prognosis. Although acetylation levels seem to be reduced more often in cancers, methylation levels may vary, depending on the type of modification or the type of cancer in consideration [60, 64]. It is also important to note that in some cases these parameters seem contradictory. For instance, in NSLCs, esophageal carcinomas and gliomas, low levels of H3K9ac or H3K18ac are associated with a better prognosis [59, 65]. Hence, the appropriate method to analyze histone marks for clinical application is to monitor a combinatorial pattern of modifications [57] and establish the prognosis depending on the cancer type [65].

In clinical studies, molecular mechanisms underlying changes in histone modification profile have not been investigated. However, knowing that many histone modifiers are mutated in cancer and

therefore may contribute to the pathology might help to explain these epigenetic changes. An attempt to propose a molecular mechanism has been provided recently by Barber and coworkers [66]. They identified SIRT7, an NAD$^+$-dependent class III deacetylase, as an enzyme that is able to deacetylate H3K18 at specific target regions and thus inhibit the expression of key cellular regulators. Loss of locus-specific H3K18ac seems then to drive the tumorigenic process and depletion of the deacetylase with siRNA shows that SIRT7 favors cancer proliferation, but not initiation [66].

Moreover, it has been shown that p300/CBP are the major enzymes responsible for acetylation of H3K18 [67, 68]. Therefore, the loss of their tumor suppressor function and their catalytic activity observed in many epithelial cancers can be the cause of the reduction of H3K18ac [24] also observed in several tissue microarrays studies. As a consequence, p300/CBP-meditated loss of H3K18ac might promote silencing of cell growth control genes [66].

Acetylation on histone lysines outside of the histone tails should also be taken into account. Acetylation of lysine 56 on histone H3 (H3K56ac) has been associated with a tumorigenic phenotype and a de-differentiated cell state [69]. This is of particular interest, considering that this mark is also involved in the DNA damage response [70, 71], therefore impairment in its distribution can affect genome stability. Novel candidates for the panel of possible biomarkers are also modifications of histone variants or the linker histone H1, which can undergo various types of modifications. The histone H1.4 variant is acetylated on lysine 34 (H1.4K34ac) and increased levels of this modification are found in seminomas [72], a type of cancer characterized by a stem cell-like phenotype.

The histone variant H2A.Z has been found to be associated with active or inactive genes with different distribution patterns, more enriched at transcriptional start site or widespread along the promoter, respectively [73]. In prostate cancer cell lines, a global reduction of H2A.Z acetylation along with a global redistribution of H2A.Z variant has been observed [73]. In particular, genomic regions that become upregulated in cancer gain H2A.Z acetylation, whereas genes that are downregulated lose the acetylated H2A.Z variant.

Evaluation of the epigenome of cancer cells could be useful to identify novel drug targets. Global H3 acetylation changes have been reported in blast cells from AML patients [74]. More than 1000 genomic loci lost their general acetylation, a phenomenon that affects mainly core promoters and is accompanied by increased DNA methylation and transcriptional repression. With this approach, novel tumor suppressor genes were discovered, which can help to explain the mechanisms underlying the pathological transformation.

This epigenetic scenario that has been delineated in the past few years proposes a model according to which analysis of histone modifications can reveal the physiology of the cells and discriminate between healthy and sick cells. Among them, histone acetylation plays a leading role, as it seems to regulate chromatin dynamics and gene expression. More importantly, this post-translational modification is the most promising from the clinical point of view, because it is so far the easiest to manipulate in disease and many approaches for epigenetic therapy have been proven to be successful.

MANIPULATION OF HISTONE ACETYLATION: A PROMISE FOR EPIGENETIC THERAPY

Unlike genetic mutations, which are stably inherited by daughter cells, epi-mutations, e.g. altered patterns of histone acetylation, can be reversible. To compensate for a reduction in global histone acetylation seen in many clinical cases of cancer and to reactivate genes that have been silenced, HDACi can be used. In many cancers altered HDAC expression rather than mutation has been reported [28, 75]. Aberrant recruitment of HDACs by fusion proteins or misregulated oncogenes is considered to be responsible for many alterations in acetylation patterns (Figure 3). Therefore, inhibiting those enzymes could help to regain a physiological epigenetic condition. Indeed, two HDACi, SAHA (Vorinostat) and romidepsin, are already granted by FDA approval for the treatment of cutaneous T-cell lymphoma and many others are in pre-clinical or clinical phase for the treatment of several types of human cancers [76]. An overview about HDACs and HDACi can be found in Khan and La Thangue [77].

Although HDAC inhibition is the first line of investigation in epigenetic therapy, attempts to establish alternative approaches are ongoing, in order to embrace several diverse clinical cases.

An interesting new research direction focuses on the use of epigenetic multiple ligands (epi-MLs),

versatile compounds bearing chemical groups active against more than one class of epigenetic modifiers [78]. Psammaplins, for example, are natural compounds that can inhibit both HDACs and DNA methyltransferases (DNMTs) [79] and their potential anticancer activity has been shown in human cancer cell lines [80]. As previously mentioned, usually a combinatorial pattern of different modifications is altered in cancer. Therefore, targeting multiple alterations simultaneously (e.g. both acetylation and methylation) might increase the efficacy of the treatment. The downside of these compounds as well as HDACi is their pan-inhibitor nature that might lack selectivity toward the specific target that is primarily causing the alteration and instead inhibit a whole class of enzymes. They might therefore also affect other physiological epigenetic pathways and result in side effects.

A valid alternative that has been already explored is the use of HATi, although permeability and specificity seem to be the main limitation to their use [81]. Curcumin and anacardic acid are two natural compounds with potent HAT inhibitory effect on p300 and CBP [82]. Curcumin, one of the most well-studied compounds, has been proven to sensitize various cancer cell lines to other anticancer chemicals via inhibition of the NF-κB pathway [83–86]. One limitation of the use of this compound could derive from its reduced solubility and poor permeability into cells, thereby limiting adsorption. Synthetic compounds with different inhibitory spectra have been taken into account [9], ensuring the identification of novel therapeutic strategies. An exception among the HATi is garcinol, a natural compound that shows high permeability, but low specificity. One of its derivative (LTK-14) has been selected to be highly specific against p300 with low toxicity and its effect *in vitro* and *in vivo* has been described [87]. In particular, inhibition of p300-mediated acetylation of viral proteins in HIV-infected T cells interferes with integration in the genome and multiplication of the virus [87].

An unexplored field in cancer is the use of HAT activators. To date, only few activators have been designed and the best characterized is the anacardic acid derivative CTBP, which has been shown to activate p300 *in vitro* [88]. In spite of its highly impermeable nature, effects of this compound *in vivo* have been studied using a more soluble carbon-sphere-conjugated CTBP [89]. However, its use in cancer research is still very limited.

Similarly, also deacetylase activators are yet not taken into account. For instance, the deacetylase SIRT3 has been already described as tumor suppressor [90] and it has recently shown that it is able to deacetylate the oncogenic protein Skp2, favoring its degradation [91]. In fact, acetylation of lysines in Skp2 nuclear localization sequence set by p300 promotes Skp2 stability and determines its localization in the cytoplasm, where it can be deacetylated. Therefore, reduced SIRT3 activity results in stabilization of Skp2 and increased Skp2-mediated degradation of tumor suppressor proteins, including E-cadherin, p27 and p21 [91]. Some breast and prostate cancers report high levels of Skp2 [92, 93], and an inverse correlation between the deacetylase SIRT3 and Skp2 has been observed in breast cancer [91]. Therefore appropriate modulation of the acetylation balance in this cancer via sirtuins activation could be a strategy that has not been exploited yet.

Most recently, an inhibitor of bromodomain-containing proteins has been tested in models of AML involving MLL fusion proteins. As bromodomains bind to acetylated lysine, they can recruit proteins or protein complexes to active acetylated chromatin and sustain transcriptional activation, as in the case of polymerase-associated factor complex. The BET (bromodomain extra terminal) proteins are a class of proteins associated with transcriptional machinery and featured with an N-terminal tandem bromodomain. In AML, these proteins are also associated with the MLL fusion, determining its aberrant targeting to oncogenes. The BET inhibitor GSK1210151A (I-BET151) is able to displace the BETs and secondarily the MLL fusion from the target oncogenes, therefore inducing cell cycle arrest and apoptosis [94]. Understanding the structural properties of the bromodomains and the mechanisms of their target recognition might help the compound design of this novel class of inhibitors [95].

In conclusion, today we can consider epigenetic manipulation of pathological cells as a novel strategy for therapy for cancer and even other diseases. This is possible because both genetic and epigenetic alterations of cells can be causative of a pathological process. In particular, acetylation is one of the most commonly impaired post-translational modifications, as it plays a key role in transcriptional activation. Due to the fact that acetylation is commonly altered in cancer, and that it is a reversible modification and

easy to manipulate *in vivo*, it will in the near future become a first-line tool for prognosis and therapy of diseases in which epigenetic states are distorted.

Key Points

- Genetics and epigenetics are interdependent factors in cancer biology.
- HATs are altered in several cancers and can act as tumor suppressors or oncogenes.
- Acetylation plays a key role in the regulation of cancer transformation.
- Histone modifications can be used as biomarkers in the prediction of patient survival.
- Use of HDACis and other epigenetic modulators in clinic could become an important therapeutic tool for treatment of cancer.

References

1. Williams K, Christensen J, Pedersen MT, *et al*. TET1 and hydroxymethylcytosine in transcription and DNA methylation fidelity. *Nature* 2012;**473**:343–8.
2. Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet* 2007;**8**:286–98.
3. Wang GG, Allis CD, Chi P. Chromatin remodeling and cancer, Part I: Covalent histone modifications. *Trends Mol Med* 2007;**13**:363–72.
4. Guil S, Esteller M. DNA methylomes, histone codes and miRNAs: Tying it all together. *Int J Biochem Cell Biol* 2009;**41**:87–95.
5. You JS, Jones PA. Cancer genetics and epigenetics: Two sides of the same coin? *Am J Pathol* 2012;**22**:9–20.
6. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. *Nat Rev Cancer* 2011;**11**:726–34.
7. Minucci S, Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat Rev Cancer* 2006;**6**:38–51.
8. Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. *Nature* 2007;**447**:433–40.
9. Vernarecci S, Tosi F, Filetici P. Tuning acetylated chromatin with HAT inhibitors: a novel tool for therapy. *Epigenetics* 2010;**5**:105–11.
10. Mai A, Altucci L. Epi-drugs to fight cancer: from chemistry to cancer treatment, the road ahead. *Int J Biochem Cell Biol* 2009;**41**:199–213.
11. Choudhary C, Kumar C, Gnad F, *et al*. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* 2009;**325**:834–40.
12. Kalvik TV, Arnesen T. Protein N-terminal acetyltransferases in cancer. *Oncogene* 2012, doi:10.1038/onc.2012.82.
13. Wang A, Kurdistani SK, Grunstein M. Requirement of Hos2 histone deacetylase for gene activity in yeast. *Science* 2002;**298**:1412–4.
14. Wang Z, Zang C, Cui K, *et al*. Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* 2009;**138**:1019–31.
15. Tropberger P, Schneider R. Going global: Novel histone modifications in the globular domain of H3. *Epigenetics* 2010;**5**:112–7.
16. Mujtaba S, Zeng L, Zhou M-M. Structure and acetyl-lysine recognition of the bromodomain. *Oncogene* 2007; **26**:5521–7.
17. Li B, Carey M, Workman JL. The role of chromatin during transcription. *Cell* 2007;**128**:707–19.
18. Karmodiya K, Krebs AR, Oulad-Abdelghani M, *et al*. H3K9 and H3K14 acetylation co-occur at many gene regulatory elements, while H3K14ac marks a subset of inactive inducible promoters in mouse embryonic stem cells. *BMC Genomics* 2012;**13**:424.
19. Kharchenko PV, Alekseyenko AA, Schwartz YB, *et al*. Comprehensive analysis of the chromatin landscape in Drosophila melanogaster. *Nature* 2012;**471**:480–5.
20. Ernst J, Kheradpour P, Mikkelsen TS, *et al*. Mapping and analysis of chromatin state dynamics in nine human cell types. *Nature* 2011;**473**:43–9.
21. Pokholok DK, Harbison CT, Levine S, *et al*. Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* 2005;**122**:517–27.
22. Santos-Rosa H, Caldas C. Chromatin modifier enzymes, the histone code and cancer. *European J Cancer* 2005;**41**: 2381–402.
23. Miremadi A, Oestergaard MZ, Pharoah PDP, *et al*. Cancer genetics of epigenetic genes. *Hum Mol Genet* 2007; **16**:R28–49.
24. Peifer M, Fernández-Cuesta L, Sos ML, *et al*. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. *Nat Genet* 2012;**44**:1104–10.
25. Pasqualucci L, Dominguez-Sola D, Chiarenza A, *et al*. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature* 2011;**471**:189–95.
26. Mulligan CG, Zhang J, Kasper LH, *et al*. CREBBP mutations in relapsed acute lymphoblastic leukaemia. *Nature* 2011;**471**:235–9.
27. Liu L, Zhen XT, Denton E, *et al*. ChromoHub: a data hub for navigators of chromatin-mediated signalling. *Bioinformatics* 2012;**28**:2205–6.
28. Ozdag H, Teschendorff AE, Ahmed AA, *et al*. Differential expression of selected histone modifier genes in human solid cancers. *BMC Genomics* 2006;**7**:90.
29. Pfister S, Rea S, Taipale M, *et al*. The histone acetyltransferase hMOF is frequently downregulated in primary breast carcinoma and medulloblastoma and constitutes a biomarker for clinical outcome in medulloblastoma. *Int J Cancer* 2008;**122**:1207–13.
30. Li M, Luo RZ, Chen JW, *et al*. High expression of transcriptional coactivator p300 correlates with aggressive

features and poor prognosis of hepatocellular carcinoma. *J Transl Med* 2011;**9**:5.

31. Xiao X-S, Cai M-Y, Chen J-W, *et al*. High expression of p300 in human breast cancer correlates with tumor recurrence and predicts adverse prognosis. *Chin J Cancer Res* 2011;**23**:201–7.

32. Hou X, Li Y, Luo RZ, *et al*. High expression of the transcriptional co-activator p300 predicts poor survival in resectable non-small cell lung cancers. *Eur J Surg Oncol* 2012;**38**:523–30.

33. Muraoka M, Konishi M, Kikuchi-Yanoshita R, *et al*. P300 gene alterations in colorectal and gastric carcinomas. *Oncogene* 1996;**12**:1565–9.

34. Gayther SA, Batley SJ, Linger L, *et al*. Mutations truncating the EP300 acetylase in human cancers. *Nat Genet* 2000;**24**: 300–3.

35. Iyer NG, Ozdag H, Caldas C. P300/CBP and cancer. *Oncogene* 2004;**23**:4225–31.

36. Iacobuzio-Donahue CA. Epigenetic Changes in Cancer. *Annu Rev Pathol Mech Dis* 2009;**4**:229–49.

37. Kalkhoven E. CBP and p300: HATs for different occasions. *Biochem Pharmacol* 2004;**68**:1145–55.

38. Tang Y, Zhao W, Chen Y, *et al*. Acetylation Is Indispensable for p53 Activation. *Cell* 2008;**133**:612–26.

39. Avvakumov N, Côté J. The MYST family of histone acetyltransferases and their intimate links to cancer. *Oncogene* 2007;**26**:5395–407.

40. Gorrini C, Squatrito M, Luise C, *et al*. Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response. *Nature* 2007; **448**:1063–7.

41. LLeonart ME, Vidal F, Gallardo D, *et al*. New p53 related genes in human tumors: significant downregulation in colon and lung carcinomas. *Oncol Rep* 2006;**16**:603–8.

42. Ferrari R, Pellegrini M, Horwitz GA, *et al*. Epigenetic reprogramming by adenovirus e1a. *Science* 2008;**321**: 1086–8.

43. Liu X, Marmorstein R. Structure of the retinoblastoma protein bound to adenovirus E1A reveals the molecular basis for viral oncoprotein inactivation of a tumor suppressor. *Genes Dev* 2007;**21**:2711–6.

44. Diab A, Zickl L, Abdel-Wahab O, *et al*. Acute myeloid leukemia with translocation t(8;16) presents with features which mimic acute promyelocytic leukemia and is associated with poor prognosis. *Leuk Res* 2013;**37**:32–6.

45. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. *Nat Rev Cancer* 2007;**7**:823–33.

46. Chaffanet M, Gressin L, Preudhomme C, *et al*. MOZ is fused to p300 in an acute monocytic leukemia with t(8;22). *Genes Chromosomes Cancer* 2000;**28**:138–44.

47. Rozman MA, Cam s M, Colomer D, *et al*. Type IMOZ/ CBP (MYST3/CREBBP) is the most common chimeric transcript in acute myeloid leukemia with t(8;16)(p11;p13) translocation. *Genes Chromosomes Cancer* 2004;**40**:140–5.

48. Wang L, Gural A, Sun X-J, *et al*. The leukemogenicity of AML1-ETO is dependent on site-specific lysine acetylation. *Science* 2011;**333**:765–9.

49. Deguchi K, Ayton PM, Carapeti M, *et al*. MOZ-TIF2-induced acute myeloid leukemia requires the MOZ nucleosome binding motif and TIF2-mediated recruitment of CBP. *Cancer Cell* 2003;**3**:259–71.

50. Yang X-J, Ullah M. MOZ and MORF, two large MYSTic HATs in normal and cancer stem cells. *Oncogene* 2007;**26**: 5408–19.

51. Kitabayashi I, Aikawa Y, Nguyen LA, *et al*. Activation of AML1-mediated transcription by MOZ and inhibition by the MOZ-CBP fusion protein. *EMBO J* 2001;**20**: 7184–96.

52. Debes JD, Sebo TJ, Lohse CM, *et al*. P300 in prostate cancer proliferation and progression. *Cancer Res* 2003;**63**:7638–40.

53. Santer FR, Hoschele PPS, Oh SJ, *et al*. Inhibition of the acetyltransferases p300 and CBP reveals a targetable function for p300 in the survival and invasion pathways of prostate cancer cell lines. *Mol Cancer Ther* 2011;**10**: 1644–55.

54. Fan S, Ma YX, Wang C, *et al*. P300 modulates the BRCA1 inhibition of estrogen receptor activity. *Cancer Res* 2002;**62**: 141–51.

55. Hobbs CA, Wei G, DeFeo K, *et al*. Tip60 protein isoforms and altered function in skin and tumors that overexpress ornithine decarboxylase. *Cancer Res* 2006;**66**: 8116–22.

56. Fraga MF, Ballestar E, Villar-Garea A, *et al*. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat Genet* 2005;**37**: 391–400.

57. Seligson DB, Horvath S, Shi T, *et al*. Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* 2005;**435**:1262–6.

58. Manuyakorn A, Paulus R, Farrell J, *et al*. Cellular histone modification patterns predict prognosis and treatment response in resectable pancreatic adenocarcinoma: Results from RTOG 9704. *J Clin Oncol* 2010;**28**:1358–65.

59. Barlési F, Giaccone G, Gallegos-Ruiz MI, *et al*. Global histone modifications predict prognosis of resected non small-cell lung cancer. *J Clin Oncol* 2007;**25**:4358–64.

60. Park YS, Jin MY, Kim YJ, *et al*. The global histone modification pattern correlates with cancer recurrence and overall survival in gastric adenocarcinoma. *Ann Surg Oncol* 2008;**15**:1968–76.

61. Mosashvilli D, Kahl P, Mertens C, *et al*. Global histone acetylation levels: Prognostic relevance in patients with renal cell carcinoma. *Cancer Sci* 2010;**101**:2664–9.

62. Kurdistani SK. Histone modifications in cancer biology and prognosis. *Progress in Drug Research* 2011;**67**:91–106.

63. Wei Y, Xia W, Zhang Z, *et al*. Loss of trimethylation at lysine 27 of histone H3 is a predictor of poor outcome in breast, ovarian, and pancreatic cancers. *Mol Carcinog* 2008; **47**:701–6.

64. Seligson DB, Horvath S, McBrian MA, *et al*. Global levels of histone modifications predict prognosis in different cancers. *Am J Pathol* 2009;**174**:1619–28.

65. llgrabe JFU, Kavanagh E, Joseph B. Histone onco-modifications. *Oncogene* 2011;**30**:3391–403.

66. Barber MF, Michishita-Kioi E, Xi Y, *et al*. SIRT7 links H3K18 deacetylation to maintenance of oncogenic transformation. *Nature* 2012;**487**:114–8.

67. Horwitz GA, Zhang K, McBrian MA, *et al*. Adenovirus small e1a alters global patterns of histone modification. *Science* 2008;**321**:1084–5.

68. Jin Q, Yu L-R, Wang L, *et al*. Distinct roles of GCN5/PCAF-mediated H3K9ac and CBP/p300-mediated H3K18/27ac in nuclear receptor transactivation. *EMBO J* 2011;**30**:249–62.

69. Das C, Lucia MS, Hansen KC, *et al*. CBP/p300-mediated acetylation of histone H3 on lysine 56. *Nature* 2009;**459**:113–7.

70. Masumoto H, Hawke D, Kobayashi R, *et al*. A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. *Nature* 2005;**436**:294–8.

71. Tjeertes JV, Miller KM, Jackson SP. Screen for DNA-damage-responsive histone modifications identifies H3K9Ac and H3K56Ac in human cells. *EMBO J* 2009;**28**:1878–89.

72. Kamieniarz K, Izzo A, Dundr M, *et al*. A dual role of linker histone H1.4 Lys 34 acetylation in transcriptional activation. *Genes Dev* 2012;**26**:797–802.

73. Valdés-Mora F, Song JZ, Statham AL, *et al*. Acetylation of H2A.Z is a key epigenetic modification associated with gene deregulation and epigenetic remodeling in cancer. *Genome Res* 2012;**22**:307–3.

74. Agrawal-Singh S, Isken F, Agelopoulos K, *et al*. Genome-wide analysis of histone H3 acetylation patterns in AML identifies PRDX2 as an epigenetically silenced tumor suppressor gene. *Blood* 2012;**119**:2346–57.

75. Kanwal R, Gupta S. Epigenetic modifications in cancer. *Genes Chromosomes Cancer* 2011;**81**:303–11.

76. Dell'aversana C, Lepore I, Altucci L. HDAC modulation and cell death in the clinic. *Exper Cell Res* 2012;**318**:1229–44.

77. Khan O, La Thangue NB. HDAC inhibitors in cancer biology: emerging mechanisms and clinical applications. *Nat Med* 2011;**90**:85–94.

78. Mai A, Cheng D, Bedford MT, *et al*. Epigenetic multiple ligands: Mixed histone/protein methyltransferase, acetyltransferase, and class III deacetylase (sirtuin) inhibitors. *J Med Chem* 2008;**51**:2279–90.

79. Piña IC, Gautschi JT, Wang G-Y-S, *et al*. Psammaplins from the sponge Pseudoceratina purpurea: inhibition of both histone deacetylase and DNA methyltransferase. *J Org Chem* 2003;**68**:3866–73.

80. Ahn MY, Jung JH, Na YJ, *et al*. A natural histone deacetylase inhibitor, Psammaplin A, induces cell cycle arrest and apoptosis in human endometrial cancer cells. *Am J Pathol* 2008;**108**:27–33.

81. Varier RA, Swaminathan V, Balasubramanyam K, *et al*. Implications of small molecule activators and inhibitors of histone acetyltransferases in chromatin therapy. *Biochem Pharmacol* 2004;**68**:1215–20.

82. Selvi RB, Kundu TK. Reversible acetylation of chromatin: implication in regulation of gene expression, disease and therapeutics. *Biotechnol J* 2009;**4**:375–90.

83. Dhillon N, Aggarwal BB, Newman RA, *et al*. Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clin Cancer Res* 2008;**14**:4491–9.

84. Aoki H, Takada Y, Kondo S, *et al*. Evidence that curcumin suppresses the growth of malignant gliomas in vitro and in vivo through induction of autophagy: Role of Akt and extracellular signal-regulated kinase signaling pathways. *Mol Pharmacol* 2007;**72**:29–39.

85. Kunnumakkara AB, Guha S, Krishnan S, *et al*. Curcumin potentiates antitumor activity of gemcitabine in an ortho-topic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor- B-regulated gene products. *Cancer Res* 2007;**67**:3853–61.

86. Sen GS, Mohanty S, Hossain DMS, *et al*. Curcumin enhances the efficacy of chemotherapy by tailoring p65NFκB-p300 cross-talk in favor of p53-p300 in breast cancer. *J Biol Chem* 2011;**286**:42232–47.

87. Mantelingu K, Reddy BAA, Swaminathan V, *et al*. Specific inhibition of p300-HAT alters global gene expression and represses HIV replication. *Chem Biol* 2007;**14**:645–57.

88. Balasubramanyam K, Swaminathan V, Ranganathan A, *et al*. Small molecule modulators of histone acetyltransferase p300. *J Biol Chem* 2003;**278**:19134–40.

89. Selvi BR, Cassel J-C, Kundu TK, *et al*. Tuning acetylation levels with HAT activators: Therapeutic strategy in neuro-degenerative diseases. *Am J Pathol* 2010;**1799**:840–53.

90. Finley LWS, Haigis MC. Metabolic regulation by SIRT3: implications for tumorigenesis. *Trends Mol Med* 2012;**18**:516–23.

91. Inuzuka H, Gao D, Finley LWS, *et al*. Acetylation-dependent regulation of Skp2 function. *Cell* 2012;**150**:179–93.

92. Traub F, Mengel M, Lück HJ, *et al*. Prognostic impact of Skp2 and p27 in human breast cancer. *Breast Cancer Res Treat* 2006;**99**:185–91.

93. Yang G, Ayala G, De Marzo A, *et al*. Elevated Skp2 protein expression in human prostate cancer: association with loss of the cyclin-dependent kinase inhibitor p27 and PTEN and with reduced recurrence-free survival. *Clin Cancer Res* 2002;**8**:3419–26.

94. Dawson MA, Prinjha RK, Dittmann A, *et al*. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature* 2012;**478**:529–33.

95. Filippakopoulos P, Picaud S, Mangos M, *et al*. Histone recognition and large-scale structural analysis of the human bromodomain family. *Cell* 2012;**149**:214–31.