Master thesis:
The role of lysine acetylation, histone acetyltransferases and bromodomain containing proteins in cancer development

Molecular Cancer Research
Chromatin and transcription

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Abstract

Histone lysine acetylation is a key regulator of gene expression. Cancer manifests because of both genetic and epigenetic alterations. In several solid tumours and haematological malignancies the histone acetylation patterns are distorted as a result of various genetic or epigenetic changes. Reduced activity of histone lysine acetyltransferases (HATs) and/or increased activity on the wrong targets contribute to the development and progression of oncogenic transformation. Bromodomain containing proteins which recognize the histone acetylation marks are mistargeted or overexpressed in some cancer types. So far, a number of epidrugs has been designed to target the epigenetic modulators of histone lysine acetylation. Further investigation of the histone lysine acetylation and deacetylation pathways and their involvement in tumorigenesis will allow the development of more efficient epigenetic therapeutic approaches for specific cancer types.

Background

Chromatin is the macromolecular complex of DNA and proteins that promotes DNA packaging in the cell nucleus. The organizing repeating unit of chromatin is the nucleosome, which consists of ~146 DNA base pairs wrapped around a histone octamer containing two copies of each of the four core histones H3, H4, H2A, H2B (1). Linker DNA of variable lengths connects the nucleosomes. Histone H1 binds to the linker DNA facilitating the stabilization of compact 30nm fibers and the formation of higher-order chromatin structure (2) (Figure 1). The accessibility for binding proteins determines the conformation of chromatin. An "open" or accessible state is referred to as "euchromatic", while a "closed" or less accessible state is "heterochromatic" (3). DNA based processes, including transcription, repair and replication require changes in chromatin's structure (chromatin remodelling) to allow access of the compact DNA to the regulatory factors that mediate them (4). Chromatin and its regulation is an example of epigenetics. Epigenetics is the study of heritable changes of DNA structure that do not alter the primary DNA sequence (5). Epigenetic mechanisms such as DNA methylation, post-translational histone modifications, nucleosome remodelling, histone variants exchange and non-coding RNAs regulate chromatin's structure in a dynamic manner (6).

Cancer was initially defined as a disease caused by the accumulation of genetic mutations which influences gene expression and results in upregulated cell growth. Nowadays it is clear that cancer manifests because of both genetic and epigenetic alterations (7). Lately it was suggested that the interplay between genetic and epigenetic mechanisms promotes tumorigenesis. Changes in epigenetic mechanisms can result in genetic mutations (eg. hypermethylation of the promoters of classic tumour suppressor genes causes their loss of function and increases their susceptibility to genetic mutations), while genetic mutations of epigenetic regulators can lead to a distorted epigenetic "landscape" (eg. mutations of epigenetic modifying enzymes may result in their aberrant activity) (8). Epigenetic alterations have been reported in both initiation and cancer progression (9). The regulatory pathways involved in the establishment of the epigenetic state of malignant cells are still not completely elucidated. The reversible nature of epigenetic changes and particularly of the post-translational histone modifications makes them promising therapeutic targets for cancer treatment. Therefore, there is great interest in the field of epigenetics related to cancer. The primary concern is understanding the epigenetic pathways that are distorted in cancer and then developing more efficient therapeutic approaches.

The first part of this review introduces general information on the different histone modifications as well as the epigenetic aspect of cancer. The second part is focused on histone lysine acetylation and its involvement in cancer development. DNA methylation pathways are also involved in oncogenesis but this subject is beyond the scope of this review (recommended review: (10)).

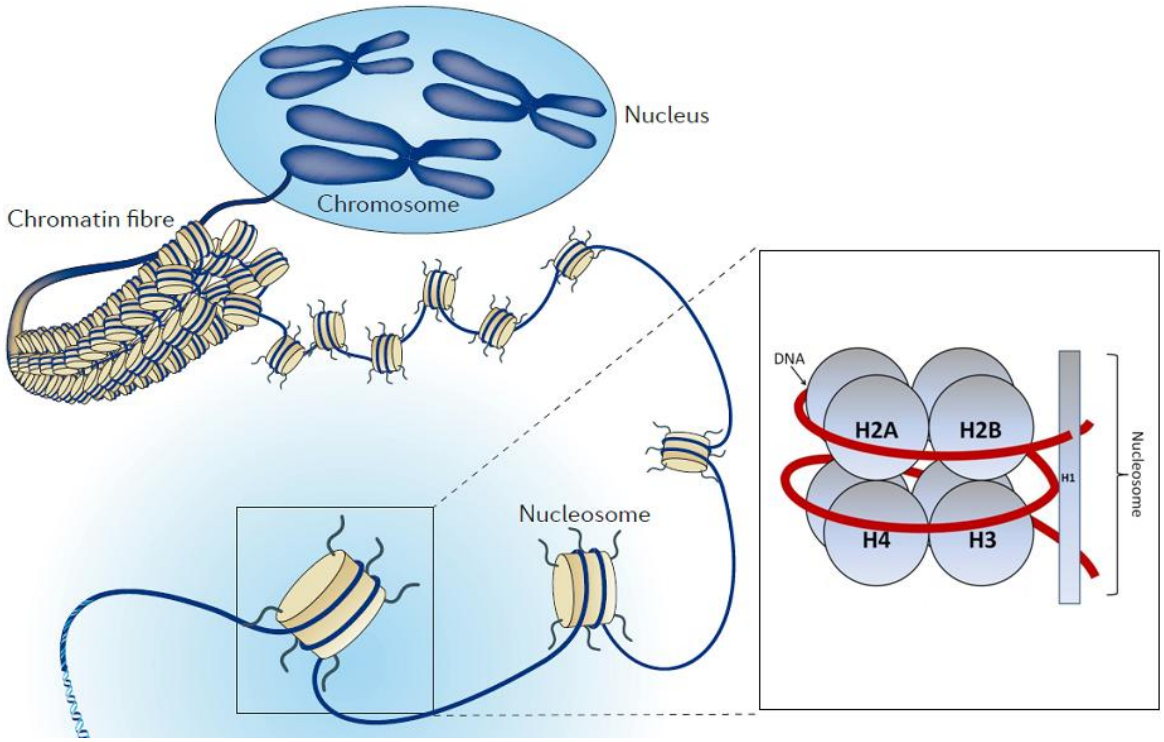

Figure 1: DNA is packaged in the cell nucleus into chromatin. The organizing unit of chromatin is the nucleosome which consists of ~146 DNA base pairs wrapped around a histone octamer containing two copies of each of the four core histones H3, H4, H2A, and H2B. Nucleosomes are connected with linker DNA. Histone H1 binds to the linker DNA and facilitates the stabilization of compact chromatin fibers. (figure adapted from Arrowsmith et al, 2012)

Post-translational histone modifications

Histone modifications and their function

The core histones are basic proteins composed of a globular domain and an unstructured N-terminal tail which protrudes from the nucleosome (11). Histones and mainly their tails are subject to a wide variety of covalent post-translational modifications. Currently, at least 16 different classes of histone modifications have been identified located in more than 60 distinct residues (12,13). Methylation of lysine/arginine residues and acetylation of lysine residues are the most well studied histone modifications. The diversity of histone modifications and the unlimited amount of their combinations result in a number of different biological outcomes.

Histone modifications have a role in a diverse array of nuclear processes. In collaboration with other proteins they are responsible for eu- and heterochromatin formation. Also they play an important role in the DNA damage response, DNA replication and transcription (12). They mediate their functions via two distinct molecular mechanisms. On one hand, modifications can directly modulate chromatin structure locally or globally by altering the histone-histone interactions or the histone-DNA interactions. For example, lysine acetylation neutralizes the lysine's positive charge resulting in weak inter-nucleosomal interactions and in a more "open" chromatin form. Likewise, phosphorylation adds negative charge to the histones that affects the inter- or intra-nucleosomal contacts. On the other hand, histone modifications serve as a signalling platform to recruit or occlude effector proteins; usually multivalent chromatin associated factors (3). These factors specifically recognize modifications via unique domains. They possess enzymatic activities such as remodelling ATPases and following their binding they can further modify chromatin.

The histone code hypothesis

Allis and colleagues (14) introduced the idea of the "histone code." The histone code is an epigenetic system involving different combinations of histone modifications ('signs") that regulate distinct downstream biological outcomes of eukaryotic genomes ('meanings") (15). Histone modifying enzymes add ("writing") and remove ("erasing") the chemical modifications. Histone acetyltransferases (HATs or KATs) add acetyl groups, while histone methyltransferases (KMTs) methyl groups. HATs and KATs are known as the "writers of the histone code. The antagonistic group of "writers", the "erasers", is responsible for the removal of the covalent modifications. So, histone deacetylases (HDACs or KDMs) remove acetyl groups and histone

lysine demethylases (KDMs) methyl groups (16). The "readers" of the histone marks are multicomponent protein complexes containing binding or reader domains such as plant homeodomain (PHD), tudor, chromo or bromo domains which recognize specific modified residues and interpret them (17).

Histone modifications interplay each other (histone modification cross-talk). One mark can influence others positively or negatively via several mechanisms. An example of histone modification cross-talk is the following; heterochromatin protein 1 (HP1) binds to di- and tri-methylated K9 residues of H3 (H3K9me2/3). During mitosis H3S10 is phosphorylated and inhibits HP1 from binding to H3K9me2/3 (3). Depending on the combination of the modifications and their location the biological output differs. For instance, methylation is correlated with both transcriptional activation and repression depending on the modified residue and the modification state (mono-, di-, tri-methylated) (12).

The new histone modifications that are being identified and the unlimited amount of their combinations establish a complex communication network (3,18). To add to the complexity it is likely that histone marks are interpreted in a context-dependent manner based on the chromatin region that they are sited and the cell signalling conditions (15,19). Therefore, the histone code is not "strict" since histone modifications do not always present the same pattern and do not carry the same meaning (19,20).

Cancer as an epigenetic disease

The epigenetic "landscape" of normal, healthy cells is the following: heterochromatic regions, such as repetitive genomic sequences are heavily methylated and bear repressive histone modification marks, including trimethylation of K27 of H3 (H3K27me3), dimethylation of K9 of H3 (H3K9me2) and trimethylation of K20 of H4 (H4K20me3). The promoters of silenced genes, for instance oncogenes, are also marked with repressive modifications, mainly H3K27me3. A gene promoter programmed for active transcription eg. tumour suppressors genes is characterized by enrichment in acetylated H3 and H4 lysine residues (K5,K8,K9,K12 and K16), trimethylation of lysine residue 4 on histone H3 (H3K4me3), the presence of the H2A.Z histone variant in proximity to gene promoters (21) and H3K36me3 in the gene body to promote productive transcription elongation (7,22,23). Enhancers are distal sequence elements that activate transcription after the binding of specific transcription factors and they are characterized by the presence of H3K4me1 (24). The balanced combination of these active and repressive signatures ensures the coordinated expression of specific genes as well as chromosomal integrity in normal cells.

This balance is disrupted in cancer cells. They are described by global DNA hypomethylation in regions that normally remain silenced. Promoters of tumour suppressor genes show hypermethylation of CpG islands and global loss of monoacetylated and trimethylated K16 and K20 of H4 respectively (25,26). In addition, tissue specific histone modifications aberrations have been reported. For instance, loss of H3K4me2 and H3K18ac are associated with higher risk of prostate cancer recurrence (27). Solid tumours and haematological malignacies present different epigenetic signatures (26). Summarizing, aberrant epigenetic mechanisms can lead to cancer phenotypes by deregulating the expression programmes of oncogenes and/or tumour suppressors or by distorting the genomic integrity.

A possible cause of this change in the chromatin "landscape" of malignant cells is the altered expression or abnormal activity of the histone modifying enzymes. Moreover, histones modifiers usually interact with other cellular components and mainly gene specific transcription factors, so upstream signals are likely to promote their involvement in aberrant gene expression programmes (28). Unlike the static genetic lesions that are responsible for numerous cancer phenotypes, in case of epigenetic alterations there is potential for reversibility. This unique characteristic gives opportunities to develop an increasing number of small molecules, named "epidrugs", which target such reversible epigenetic changes (28)(9). Histone acetylation and methylation pathways are logical targets for these drugs since they are the most widely distorted among the histone modifications in human cancers (16,29).

Histone lysine acetylation

Lysine acetylation occurs when an acetyl group from acetyl-CoA is transferred to the ε-amino group of a lysine residue (N^{ε} –acetylation) and is distinguished from protein N-terminal acetylation (N^{α}-acetylation) (30). The presence of acetyl-groups on histones was first reported by Phillips et al in the early 1960s (31). Acetylation on histones in cells is maintained at required levels by the antagonistic activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs) that catalyse the acetylation reaction (32). All of the core histones can be acetylated on different lysine residues of the N-terminal tails (33,34). The acetylation sites are more conserved in histone H3 and H4 and less conserved in histones H2A and H2B (35). Acetylation of H3K9, H3K14 and H3K18 is correlated with active transcription (35,36). H4K16 acetylation is also associated with transcription activation and the maintenance of euchromatin (37).

Initially it was believed that histones are the only substrates for acetylatransferases. Later, however it was revealed that acetyltransferases besides the histones can acetylate a wide range of non-histone proteins: DNA-binding transcription factors and other nuclear proteins (eg. transcriptional co-regulators, general transcription factors), non-nuclear proteins (a-tubulin) and proteins that are transferred between the nucleus and the cytoplasm (importin α) (30,38). The acronyms HATs and HDACs are still used, but their meanings are slightly different from the originals that referred only to histones. Lysine acetylation is now considered as a general post-translational modification that modulates numerous cellular proteins. It has been compared to phosphorylation because of their common substrates diversity and the variety of their biological functional consequences (39).

Histone lysine acetylation and transcription

Histone acetylation has been implicated in the regulation of chromatin-based nuclear processes including gene expression (12). Hence, it is an important regulator of different cellular and developmental programs. The involvement of histone lysine acetylation in transcription regulation was early suggested after the observation that histones are hyperacetylated in actively transcribed chromatin regions and hypoacetylated in transcriptionally silent regions (40). In accordance with this is the fact that acetylated residues are mainly sited in promoters and enhancers of active genes and in some cases throughout the gene body (9).

Histone acetylation regulates gene expression via two distinct mechanisms. On one hand, the acetylation of a histone lysine residue reduces the positive charge of histones and consequently the electrostatic interaction between histones and negatively charged DNA are disrupted. This results in a more "open" chromatin conformation that increases DNA accessibility for the multiprotein complexes that mediate transcription. In addition to the altering of the electrostatic interactions, histone acetylation serves as a docking site for effector proteins (41). For instance, the Swi2/Snf2 chromatin-remodelling complex contains bromodomains that bind acetylated lysine. Subsequently it remodels the targeted chromatin region to a more "relaxed" form (3).

In contrast with acetylation, histone deacetylation is associated with transcriptional repression by promoting chromatin condensation. However, in some cases HDACs can also work as transcriptional activators. For example, the Hos2 yeast deacetylase is necessary for efficient gene activity. It was shown that Hos2 binds to the coding regions of highly activated genes and deacetylates lysines residues of histone H3 and H4. Probably the Hos2 deaceatylation activity is required to reverse the transcriptional activated chromatin within the coding region to its initial state so that multiple transcription cycles are allowed (42).

It was already mentioned that histone modifications communicate with each other. This is also the case for histone lysine acetylation. For instance, the lysine 9 of H3 can be either acetylated or methylated resulting to an "open" or "closed" chromatin structure respectively. Since the acetylation and methylation are mutually exclusive on one lysine residue HDAC activity is necessary to switch between H3K9ac and H3K9me3. Subsequently, methyltransferases come in to add methyl groups and promote heterochromatin formation (32). Another example of histone lysine acetylation crosstalk with other epigenetic mechanisms is HDACs interaction with the DNA methylation machinery. It has been shown that proteins with methyl DNA-binding activity can recruit multisubunit complexes containing HDAC activity, thereby promoting gene silencing (34).

Writers, erasers and readers of histone lysine acetylation

Three major families of HATs (writers of histone acetylation) are: GNATs (general control non-derepressible 5 (Gcn5)-related N-acetyltranferases), p300/CBP (adenoviral E1A-associated protein of 300kD)/ (CREB-binding protein) and MYST (for the founding members MOZ, Ybf2/Sas3, Sas2 and Tip60). The above families comprise the nuclear or A-type HAT proteins that are distinguished from the cytoplasmic or B-type HATs. Although the different groups carry out the same reaction they exhibit significant sequence diversity probably associated with their discrete substrates and the biological processes that they are involved (43).

The HDAC (erasers) superfamily comprises of four family proteins: Class I, II, III and IV subdivided according to their homology to yeast HDACs. Class I (homologous to Rpd3) includes HDAC 1, 2, 3, 8. They are localized in the nucleus and they are ubiquitously expressed. Class II (homologous to Hda1) consists of two subgroups: IIa including HDAC 4, 7, 9 and IIb including HDAC 6 and 10. These HDACs are found both in the nucleus and the cytoplasm and their expression patterns are tissue-specific. Recently, HDAC 11 was identified and is the only member of class IV. It is nuclear specific and presents sequence similarities with class I and II. Class III, also named sirtuins, comprises of a group of proteins SIRT1-7 homologous with the yeast Sir2 family. Some of them are nuclear, while others cytoplasmic. All deacetylases share a highly conserved deacetylase domain and they are Zn^{2+} dependent, except from Class III that is NAD+ dependent (44,45). HATs and HDACs are usually components of large multisubunit protein complexes *in vivo*. The non-catalytic subunits of these complexes determine the specific activity as well as the substrate specificity of the catalytic subunits (32).

Acetylated residues are mainly recognized by bromodomains and tandem PHD domains (17). Usually the bromodomains exist in big complexes

together with other chromatin-associated factors. At least 42 different human proteins possess a bromodomain or tandem bromodomains. They can be classified into nine major families based on their sequence similarities. Although the different members do not present high sequence similarity, they all share a highly conserved motif the BRD fold, which binds to the acetyl groups (46).

Abnormal activity of writers, erasers or readers of histone lysine acetylation may distort the normal histone acetylation patterns and interpretation of them leading to carcinogenesis (Figure 2). The following sections discuss the link of HATs and bromodomain containing proteins with cancerogenesis. HDACs are also involved in cancer (44) but they are not discussed in this review.

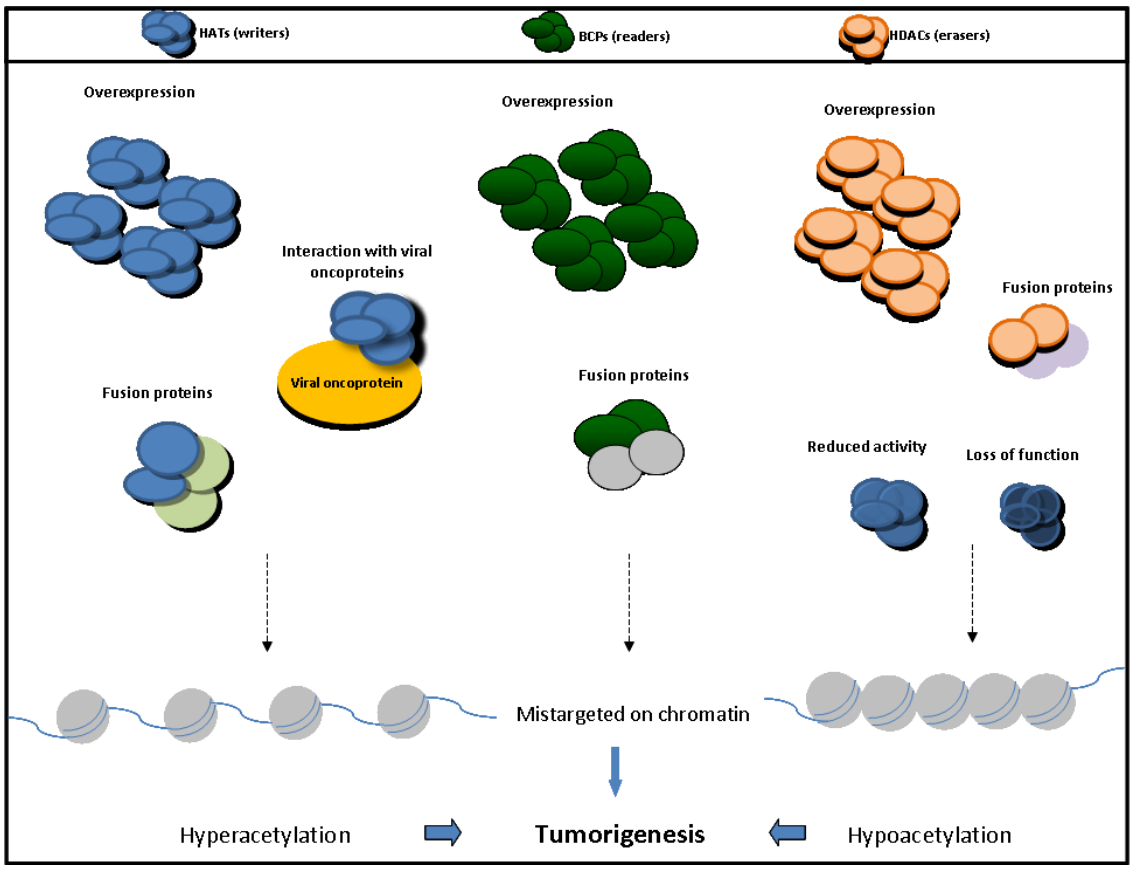

Figure 2: Aberrant activity of histone acetyltransferases or histone deacetylaces alters the normal histone acetylation levels and results in tumorigenesis. Bromodomain containing proteins are also involved in cancer development.

HATs involved in cancer development

p300/CBP family

p300 was at first identified as the binding partner of the adenovirus early region 1A (E1A) protein. It is highly homologous (63% identical) to the cyclic AMP response element binding (CREB) protein (CBP) as they share numerous conserved regions that contain mainly the functional domains of these proteins. Besides their high homology the two proteins perform both overlapping and unique functions (47). Before the discovery of their HAT activity, p300 and CBP were characterized as global transcriptional co-activators. Among their targets are oncoproteins (eg. myb, jun, fos), transforming viral proteins (eg. E1A, E6 and T-large antigen) and tumour suppressor proteins (eg. p53, E2F, Rb, Smads, RUNX and BRCA1). Regarding their histone acetyltransferase activity they can add acetyl groups at several lysine residues of all four histones. Apart from their histone substrates, they can also acetylate at least 70 other non-histone proteins, including p53, p73, Rb, E2F, myb (48). p300/CBP are implicated in several cellular processes: cell-cycle regulation, proliferation, differentiation, apoptosis and DNA damage response (47). Given their involvement in such critical biological pathways, mutations or altered expression patterns of HAT genes can contribute or even lead to oncogenesis. In fact, genetic defects of p300 and CBP (Table 1) have been identified in solid tumours and haematological malignancies (47). Both p300 and CBP have been considered to act as putative tumour repressor genes. Their suppressive activity is further confirmed by mouse experiments in which double null embryonic stem (ES) cells for p300 and CBP (-/-) injected into blastocysts induced haematological malignancies in the chimeric mice (47).

Coding mutations

Early analysis of primary tumours and cancer cell lines confirmed p300 involvement in tumorigenic process and its tumour suppressive properties. Mutations occurring with a low frequency usually accompanied by loss of the second allele were identified in breast, gastric and colorectal primary tumours (49),(50). Furthermore, mutations of p300/CBP, including deletions, insertions, truncations, missense and point mutations were detected in screenings for loss of heterozygosity (LOH), which is a common incidence for tumours suppressor genes in cancers (51). For example, analysis of 27 colon and 2 gastric carcinomas revealed two missense point mutations in EP300. Both of them concerned amino-acid substitutions at conserved residues in functional

domains of the p300: the HAT domain (colorectal carcinoma) and the cystein-histidine rich domain which mediates protein-protein interactions (gastric carcinoma), probably resulting in inactivated enzymes. In addition, loss or silencing of the second allele was observed indicating the typical two-hit hypothesis for tumour suppressor genes (34,47). The two hit hypothesis suggests that biallelic inactivation of a tumour suppressor gene results in tumorigenesis. A mutation in one allele of the tumour suppressor gene (first hit) is not sufficient for tumour development. Inactivation of the wild-type allele (second hit) because of mutations or hypermethylation leads to tumorigenesis (loss of heterozygosity). According to the aforementioned findings and the two hit hypothesis, it seems that p300 undergoes this phenomenon resulting in carcinomas of the digestive tract.

Integrative genome sequencing analyses showed that CREBBP and, less frequently, EP300 genes harbour mutations in small-cell lung cancers (52) and in non-Hodgkin B-cell lymphomas (53). Usually these mutations accumulate in the HAT coding domain leading to its inactivation or loss. Moreover, they are mainly heterozygous, suggesting haploinsufficiency. Likewise, impaired enzymatic activity caused by mutations in the HAT domain of CREBBP have been referred in 18,3% of acute lymphoid leukaemia (ALL) patients (54). Heterozygous germline mutations of one CREBBP allele that inactivate its catalytic activity is linked to the Rubinstein-Taybi syndrome (RTS), a congenital, developmental disorder with increased susceptibility for developing brain and neuronal tumors during childhood (47),(55). The majority of the RTS patients (61%) bear mutations in the CREBBP gene. Mutations in the EP300 gene have been also reported as a causative reason for RTS but they are not so common (55). CBP mutations have also been detected in human esophageal squamous cell carcinoma and in lung cancer (55). Finally, mutations of p300/CBP have been observed in leukaemias but they are rather rare (56).

Overall, loss of function of p300 and less commonly of CBP because of mutations is reported in a wide variety of solid tumors and hematological malignancies (Table 1).

Altered expression profiles

Besides the point mutations that result in loss of the HAT enzymatic activity, altered expression of p300/CBP may contribute to oncogenesis usually because of aberrant acetylation. Actually breast cancer, hepatocellular carcinoma, non-small lung cancer and prostate cancer have been shown to have p300 overexpression (51).

Chromosomal translocations

Both CREBBP and EP300 have been implicated in chromosomal rearrangements present in a wide range of haematological malignancies. The

novel protein product usually possesses the HAT catalytic domain of one partner and the DNA-binding domain of the other. As a consequence HAT activity at specific genomic sites is lost causing possible repression of normally expressed genes, mistargeted acetylation that may lead to activation of usually silent genes and sequestration of key nuclear factors that are likely to promote tumorogenesis (57).

The monocytic leukemia zinc-finger protein (MOZ) fused with CBP, MOZ-CBP t(8,16)(p11,p13), gives rise to a new chimeric protein which retains the HAT catalytic activity and the bromodomain of CBP. This translocation occurs in 0,4% of the acute myeloid leukemia (AML) cases. Likewise, p300 can also fuse with MOZ, MOZ-p300 t(8,22)(p11,q13), but is not as frequent as MOZ-CBP rearrangement (30,47). p300/CBP fusions with the mixed lineage leukemia (MLL) have been identified in treatment related haematological disorders. MLL-CBP t(11,16)(q23,p13) and MLL-p300 t(11,22)(q23,q13) translocations represent only the 1% of the total MLL fusions (47,58). The chimeric product resembles in structure with the MOZ-p300/CBP fusion protein. CBP can be also rearranged with the MORF (MOZ-related factor) gene in acute myeloid leukemia cases and the fusion protein exhibits similar structural organisation to MOZ-p300 (57,59). Apparently, CBP is a more common target than p300 in chromosomal translocations. It is possible that there is an unstable genomic hotspot in that region responsible for this higher recurrence (47).

Interaction with viral oncoproteins

Transforming viral proteins such as the E1 (adenovirus), E6 (papilloma virus) and large T-antigen (SV40) interact with numerous cellular components and induce malignant cell transformation. p300/CBP are targets for these transforming viral proteins. E1a induces genome wide redistribution of p300/CBP and targets them to the promoters of a subset of genes implicated in cell cycle and growth leading to p300/CBP and H3K18ac enrichment at those sites accompanied by transcriptional activation (60,61) Consequently, viral oncoproteins interactions with HATs inhibit their tumour suppressive activity and enhance their aberrant acetyltransferase activity because of their redistribution to other genomic locations. Therefore, the tumorigenic potential of p300/CBP is promoted (51).

Acetylation of non-histone proteins and tumorigenesis

HATs can also regulate the functions of non-histone proteins. Acetylation of critical lysine residues of non-histone substrates modulates their sequence-specific DNA binding affinity, protein-protein interactions and their subcellular localization. For example in response to stress conditions, p300 and CBP acetylate the tumour suppressor p53, so that p53 is activated and stabilized (62). Therefore, impaired HAT activity or mistargeted

acetylation can alter the function of cancer related non-histone proteins and contribute to oncogenesis.

Another example in which acetylation of non-histone proteins results in cancer development is the regulation of the fusion proteins AML1-ETO and MOZ-TIF2 (transcription intermediary factor 2) by p300/CBP. The resulting product of AML1-ETO t(8,21)(q22,q22) rearrangement, the most common in AMLs, is leukemogenic only after site-specific acetylation by p300 (51). Similarly, when the MOZ-TIF2 inversion (inv(8)(p11q13) product is acetylated by the CBP becomes oncogenic (63).

The MYST family

The MYST family is a highly conserved group of proteins in eukaryotes. In humans, this family comprises of five HATs: Tip60 (HIV Tat-interacting protein of 60kDa), MOZ (monocytic leukaemia zinc-finger protein), MORF (MOZ-related factor), HBO1 (HAT bound to ORC1) and MOF (male-absent on the first). They all possess a conserved MYST domain which contains an acetyl-CoA binding motif and a zing finger essential for their HAT activity. Some members also share other conserved structural modules such as chromodomains, plant homeo domains (Phd) or a second zing finger region. They exist only in multisubunit complexes, for example with members of the ING family, which are also evolutionarily conserved (33). Compared to the rest of the HATs families, MYST is involved in a wider range of biological processes. Tip60 is considered a transcriptional co-activator of many key cellular factors including nuclear hormone receptors, c-Myc oncoprotein, E2F, Rb and p53. For example, Tip60 acetylates p53 after DNA damage. This modification stimulates the cell to enter the apoptotic pathway. In addition, Tip60 can acetylate and thereby activate the ATM (ataxia telangiectasia mutated) kinase, a vital regulator of the DNA repair pathway. So, Tip60 is associated with the DNA repair signalling. HBO1 is the only HAT that is linked to DNA replication as it facilitates pre-replication complex formation and the initiation of DNA replication. It is also likely that HBO1 plays a role during the S phase by promoting DNA synthesis throughout the elongation phase. In addition it also acts as a transcriptional regulator. Similarly, MOZ and MORF are transcriptional activators and MOZ is a crucial regulator of the developmental programme of hematopoietic stem cells (57). Their extensive role in such critical cell processes implies that abnormal activity of these HATs or their complexes can result in cancer development. Indeed, deregulated MYST HATs have been reported in several cancer types (Table 1).

Chromosomal translocations

The role of MOZ in chromosomal translocations with CBP and p300, as well as in the chromosomal inversion with TIF2 and leukemogenesis is well established and discussed before in this review. Multiple findings, such as the deregulation of AML1-mediated transcription because of the MOZ-CBP fusion protein, the number of genes whose expression is altered due to the MOZ-CBP chimera as well as the ability of MOZ-TIF2 to induce AML in irradiated mice, suggest that the presence of MOZ in chromosomal rearrangements leads to bona fide tumorigenesis. In addition, it has been reported that MOZ is a retroviral integration site resulting in myeloid/lymphoid tumours (57).

Besides MORF's implication in AML and myelodysplastic syndromes because of chromosomal rearrangements with CBP, MORF gene disruption t(10;17)(p11;q21) has been reported in several cases of benign uterine smooth muscle tumours (64).

HBO1 coding region has also been recognized as a hotspot for retroviral integration, an event that has been correlated with the manifestation of B cell lymphomas in mice (65).

Altered expression profiles

Overexpression of HBO1 has been observed in a specific subset of 11 human primary tumours with a particularly strong expression pattern in testis, ovary, breast, stomach, esophagus and bladder carcinomas (66).

MOF targets specifically the H4K16 residue. Global reduction of H4K16ac is a common hallmark of human tumours. MOF's downregulation is frequently noticed in medulloblastomas and breast carcinomas (67). Consequently, the link between MOF, H4K16ac levels and carcinogenesis is obvious. However, the molecular mechanism remains to be discovered.

Tip60 has been found significantly downregulated in colon and lung cancers (57).

Mutations

Mutations of the Tip60 locus correlated with decreased mRNA and protein Tip60 levels have been detected in head and neck squamous carcinomas, ductal breast carcinomas and lymphomas. These tumours presented LOH of the locus at single nucleotide level and more rarely aberrant CpG methylation suggesting that Tip60 acts a haploinsufficient tumour suppressor in humans (68).

Acetylation of non-histone proteins and tumorigenesis

As mentioned before some of the acetyltrasferases do not directly act as classical tumour suppressors or oncogenes but they affect other critical non-histone cancer related proteins such as p53 and Myc either by functioning as

acetyltransferases or as transcriptional co-activators. Tip60 is an example of the first. It was demonstrated that Tip60 specifically acetylates p53 upon DNA damage in its DNA binding domain at K120. Following this modification cells are led into apoptosis. Hence, it is possible that Tip60 and p53 are implicated in a tumour progression pathway where p53 acetylation by Tip60 is prevented (57) .

The MYST HAT complex subunits may be also involved in cancer pathogenesis. For instance, ING4 and 5 are known tumour suppressors and form complexes with HBO1. Therefore, any HBO1 malfunction could influence their function and lead to malignancy (57).

GNAT family

The human genome encodes for two Gcn5 like proteins: GCN5 and PCAF (p300/CBP-associated factor). Both proteins can interact with p300/CBP and they are implicated in transcriptional regulation and cell cycle control. Targets of PCAF are the p53 and EF2 proteins, important cell cycle regulators. PCAF has a dual role: promoting cell cycle progression by targeting E2F or causing cell cycle arrest by targeting p53 (34). Therefore mutations in the HAT coding or specificity region may influence cell proliferation and contribute to tumour progression.

The fact that PCAF interacts with the viral oncoprotein E1A, as does p300/CBP, suggests its possible involvement in malignant cell transformation (60). In addition, mutations of both PCAF and GCN5 have been reported in different kind of tumour types (51). PCAF is frequently downregulated in esophageal squamous cell carcinoma samples compared to healthy tissues. PCAF's downregulation was also correlated with hypermethylation of the PCAF gene promoter region indicating its tumour suppressive nature (69,70). On the other hand, PCAF and Gcn5 overexpression has been observed in central nervous system tumours and Wilm's tumours (71). Moreover, altered expression of PCAF has been reported in different cell lines (72).

Conclusion

Histone acetyltransferases are involved in a wide range of cancer types, particularly in epithelial and haematological malignancies. They have a dual role in oncogenic transformation; they can both act as tumour suppressors as well as oncogenes, according to the cellular or molecular conditions. Normally silent genes (eg. oncogenes) can be activated and normally active genes (eg. tumour suppressor) can be repressed because of abnormal HAT activity. Different kind of genetic alterations (Table 1) in critical regions of genes encoding HAT proteins such as the HAT domain results in loss of the catalytic activity or mistargeted function of the HATs. Associated proteins with HATs such as HAT complex subunits or non-histone protein targets (eg.p53, Myc) are also implicated in cancer development. Altered expression

patterns of different HATs without a causative mutation have been observed indicating the wide variety of the mechanisms by which HATs are involved in tumour progression.

Table 1: HATs in cancer (primary tumours)

HATs Families		Genetic Defect	Tumour type	Functional consequence	Ref.
p300/CBP	p300	Truncating mutation	Breast, colorectal, gastric cancer	Loss of function at specific genomic sites, mistargeted acetylation and sequestration of key nuclear factors	(49,50)
		Missense mutation + LOH	Colon and gastric cancer		(34)
		Missence mutation	MDS		(56)
		Deletions, somatic mutations	B-NHL		(53)
		MOZ-p300 gene fusion t(8,22)(p11,q13)	AML		
		MLL-p300 gene fusion t(11,22)(q23,q13)	Treatment related hematological disorders		(47),(55)
		Upregulation	Breast cancer, hepatocellular carcinoma, non-small lung cancer prostate cancer		(51)
	CBP	Truncating/missense mutations and deletions	NSLC	Loss of function	(52) (53)
		Deletions, somatic mutations	B-NHL		(55)
		Duplications; microdeletions	Eosophagel cancer		
		Heterozygous germiline deletion	RTS	Loss of function	(47)
		MOZ–CBP gene fusion t(8,16)(p11,p13)	AML;AMML	Loss of function at specific genomic sites, mistargeted acetylation and sequestration of key nuclear factors	(47)
		MLL–CBP gene fusion t(11,16)(q23,p13)	t-AML ; t-CMML		(55)
		MORF–CBP gene fusion	AML ;t-MDS		
		Point mutations; deletions	ALL	Loss of function	(54)
MYST	MOZ	MOZ-TIF2 inversion inv(8)(p11q13)	AML	Oncogenic after CBP acetylation	(51,51)
	MORF	MORF-unknonw t(10;17)(p11;q21)	Benign uterine smooth muscle tumours	Mistargeted-aberrant acetylation	(64)
	MOF	Downregulation	Medulloblastomas and breast carcinomas	Loss of function	(67)
	Tip60	Downregulation	Colon and lung cancer	Loss of function	(73)
		Mutations	Head and neck squamous carcinomas; ductal breast carcinomas; lymphomas		(68)
	HBO1	Upregulation	Testis, ovary, breast, stomach, esophagus and bladder carcinomas	Aberrant acetylation	(66)
GNAT	GCN5	Deletions; amplifications	Breast, colorectal, prostate, lung, kidney, sarcoma	Loss of function	(51)
	PCAF	Missense,frameshift, deletion,amplification	Lung, kidney, sarcoma, colorectal		
	PCAF	Downregulation	Esophageal squamous cell carcinoma		(70)
	PCAF/GCN5	Upregulation	Central nervous system tumours and Wilm's tumours	Aberrant acetylation	(71)

MDS: Myelodysplastic syndrome; B-NHL: B-cell non-Hodgkin's lymphoma; AML: acute myeloid leukemia; NSLC: small cell lung cancer; RTS: Rubinstein-Taybi syndrome; AMML: acute myelomonocytic leukemia; t-AML: therapy-related acute myeloid leukemia; t-CMML:therapy-related acute myelomonocytic leukemia; t-MDS: therapy related myelodysplastic syndrome.

Bromodomain containing proteins (BCPs) implicated in carcinogenesis

So far it was discussed how genetic alterations in the writers of histone lysine acetylation lead to cancer. This last part focuses on the readers of lysine acetylation and their implication in cancer. At the end a brief overview of the current epidrugs that target the epigenetic regulators of histone lysine acetylation is presented.

As previously mentioned a variety of nuclear multisubunit complexes including HATs, chromatin remodelers, methyltransferases and transcriptional coactivators possess bromodomains (BRDs), which target them to specific sites. Many of these complexes have been associated with the manifestation of several cancer forms (Table 2). Oncogenic translocations and upregulation of specific BRD proteins clearly illustrate their implication in tumourigenic pathways (74).

The BET family in cancer

The bromodomain and extra-terminal domain (BET) family consists of four members: the ubiquitously expressed bromodomain-containing proteins 2, 3, and 4 (BRD2, BRD3 and BRD4) and the testis-specific expressed BRDT. All members present highly conserved tandem N-terminal bromodomains and an extra-terminal module. BET proteins are implicated in transcription and cell cycle regulation. Specifically, BRD4 facilitates transcription activation and elongation after its interaction with the mediator complex and the positive transcription elongation factor b (P-TEFb) respectively. BET proteins have been also found to bind acetylated chromatin throughout mitosis, probably conferring to "gene bookmarking", a process of post-mitotic transcriptional re-activation of integral genes. The essential role of BET-proteins is underlined by the fact that Brd2 or Brd4 mice knockouts are lethal (75). Given their important functions in transcription and cell cycle regulation abnormal BET protein expression or mistargeting on chromatin may distort these processes and contribute to cancer pathogenesis (Table 2).

BRDT overexpression is frequently observed in non-small-cell lung cancer and other cancer types. However, the functional outcome of BRDT upregulation remains unidentified (74). Another member of BET family, BRD4, presents even higher oncogenic potential because of its key role in cell cycle regulation and transcriptional elongation of growth related genes (75). It has been shown that BRD4 is involved in breast cancer progression and its expression levels have been correlated with patient survival (76). BRD4, together with BRD2, are implicated in the transmission of transforming viruses by binding the viral episomes to host chromosomes during mitosis. Such an example is BRD4 interaction with latency-associated nuclear antigen

1 (LANA), a crucial regulator of Kaposi's sarcoma-associated herpes virus (KSHV) genome replication. BRD4 serves as a docking site for LANA to the host chromatin contributing to the development of KSHV-associated malignant transformation (77).

NUT midline carcinoma: NUT-BRD4/BRD3 fusion proteins

Chromosomal rearrangements of BRD4 and BRD3 are involved in rare, particularly aggressive epithelial tumours named nuclear protein in testis (NUT)-midline carcinoma (NMC) (Table 2). Morphologically NMCs are described as undifferentiated or poorly differentiated squamous cells disrupted from foci of well differentiated squamous epithelium (78,79). Tumours manifest in the midline anatomical parts and mainly in the upper aerodigestive tract and the mediastinum. The genetic basis of NMC lies in chromosomal rearrangements of the NUT gene. NUT expression is confined to postmeiotic spermatids, but its role remains unknown. Some findings demonstrate that the physiological function of NUT is correlated with its interaction with p300 followed by its activation and/or by interaction with other yet unidentified HATs (80).

Roughly two-thirds of NMC cases present a reciprocal chromosomal translocation of the NUT gene with the BRD4, BRD4-NUT t(15;19)(q13;p13.1). The rest of the incidents bear different translocations of the NUT gene (NUT-variants). BRD3 is the fusion partner of 25% of NUT-variants, whereas the remaining variants have not been identified yet (78). The BRD4-NUT and BRD3-NUT translocation products encode for chimeric proteins consisting of the BRDs functional domains including the two tandem N-terminal bromodomains and almost the entire NUT encoding region together with the acidic domain which recruits p300 (78,81).

Functional studies, including RNAi, confirm the key role of the BRD-NUT fusion proteins in NMC. BRD-NUT oncogenic chimeras impede epithelial differentiation and promote the aberrant proliferation of the undifferentiated cells (82). It has been shown that BRD-NUT binds through its bromodomains to distinct nuclear foci, the NUT partner of the chimeric protein interacts with and recruits p300 and activates its enzymatic activity. Continuous rounds of BRD4-NUT/p300 interplay result in the creation of hyperacetylated nuclear foci, but transcriptionally inactive. It is interesting that despite histone hyperacetylation at those sites chromatin is condensed and transcription is repressed. Probably, chromatin condensation depends on acetylation. The same observation was previously reported in Brdt-activity (80).

The exact mechanism of NMC has not been elucidated yet. However, it is evident that the aberrant tethering of NUT on chromatin through the bromodomains of BRD4 and BRD3 results in the oncogenic progression of

NMC. Also this is an example of a cancer phenotype in which both misregulation of the writer as well as the reader protein of acetylation lead to tumorigenesis.

BET family in haematological malignancies

Recent findings indicate involvement of the BET proteins in a wide range of haematopoietic cancers and especially MLL-translocated leukemias. It was shown that BRD3 and BRD4 interact with the superelongation complex (SEC) and the polymerase-associated factor (PAF) complex, two critical complexes for the oncogenic activity of MLL fusion proteins. Many MLL fusion partners belong to SEC, whereas PAF complex is related with the conserved N-terminal part of MLL fusions. Both of them contribute to the aberrant transcription that is observed in leukemia (75). BRD4's role in leukemia was also demonstrated by RNAi experiments in which the survival rate of MLL-AF9 leukemia was decreased both *in vitro* and *in vivo*, after BRD4 inhibition (83).

A common observation in a range of malignant hematopoietic cell lines following BET inhibition with either RNAi or specific BET inhibitors is the decreased expression levels of MYC. So it seems that there is a link between MYC and BET proteins. MYC is a transcription factor, which regulates the expression of critical genes of cell growth and proliferation, cell cycle control and apoptosis. MYC is upregulated in many cancer types usually because of gene amplification. Its aberrant expression leads to upregulated cell growth and finally to tumorigenesis. Downregulation of MYC transcription after BET inhibition was reported in MLL-translocated acute myeloid leukemia, multiple myeloma and Burkitt's lymphoma (9). A possible mechanism that explains Myc downregulation after BET inhibition is the following: BRD4 tethers to acetylated histones via its bromodomains and facilitates transcription activation of MYC and Myc-dependent target genes. BET-inhibitors prevent the binding of BRD4 on acetylated chromatin leading to downregulation of MYC transcription and its target genes (84).

Other BCPs in cancer

AAA ATPase ANCCA (AAA nuclear coregulator cancer-associated protein) also known as ATAD2 is a transcriptional coregulator of estrogen receptor alpha (ERa) and androgen receptor (AR). Both its ATPase module and the bromodomains mediate its function as a transcriptional coregulator. Recent experiments showed that ATAD2 bromodomain recognizes the H3K14ac mark and promotes the transcriptional activation of E2F targets genes, which are important regulators of cell cycle. Moreover, E2F transcriptional activation and cancer cell proliferation mediated by ATAD2 are lost after mutations in the bromodomains indicating the possible

involvement of ATAD2 bromodomain in tumorigenesis (85). ATAD2's link with MYC-depended transcription further demonstrates its oncogenic potential (86). ATAD2 aberrant expression, as well as its correlation with tumour cell proliferation (tumour histological grades) and disease progression, is frequently reported in breast tumours (>70% of cases) (85). ATAD2 is also highly expressed in prostate cancers (87). The upregulation of a related ANCCA/ATD2 protein, ATAD2B, has been observed in glioblastomas, oligodendrogliomas and breast cancer (74).

Overexpression of another bromodomain containing protein, Tripartite motif-containing 24 (TRIM24) has also been reported in breast cancers. TRIM24 is a multifunctional protein containing tandem plant homeodomain and bromodomain regions. TRIM24 PHD-bromodomain interacts with distal estrogen response elements (EREs) and stimulates estrogen-depended genes related to cellular growth and tumor progression. Their abnormal expression may lead to cancer development (88). TRIM33 which belongs to the same family with TRIM24 has been found rearranged in papillary thyroid tumors (75).

Finally, truncating mutations in the polybromodomain containing gene PBRM1 were described in renal cell carcinomas (75).

Conclusion

Taken all these data together bromodomain containing proteins are involved in oncogenic transformation. Chromosomal translocations and overexpression are common genetic alterations that are observed in cancers involving BCPs (Table 2). The causal defect that leads to cancer phenotypes does not lay in the BRD module itself, but usually to the components of the multisubunit complexes that BRDs exist in. Critical factors of the multicomponent complexes are tethered on chromatin through the bromodomains. If they are mistargeted on chromatin (translocations and transforming viruses) or they are upregulated they can contribute to oncogenesis.

Table 2: Bromodomain containing proteins (BCPs) involved in cancer

BCPs	Genetic defect	Tumour type	Ref
BRD3/BRD4	Chromosomal translocations (most common: t(15;19)(q13;p13.1)	NUT midiline carcinoma	(78)
BRD4	Upregulation	Breast cancer	(76)
BRDT	Upregulation	Non-small-cell lung cancer	(75)
ATAD2	Upregulation	Breast cancer Prostate cancer	(85) (74)
ATAD2B	Upregulation	Glioblastomas, oligodendrogliomas and breast cancer	(75)
TRIM24	Upregulation	Breast cancer	(88)

Writers, readers and erasers of histone lysine acetylation as therapeutic targets

Nowadays, the contribution of acetyl writers, readers and erasers in the progression of hematopoietic malignancies and solid tumors emerges more and more. Their dynamic nature allows their use as promising therapeutic targets. In fact, a variety of small molecules that target HATs, HDACs and bromodomains have been developed the last decade (9).

The global histone acetylation reduction as well as the overexpression and mistargeting of HDACs that is observed in many cancers led to the design of the first HDAC inhibitors (HDACi). The inhibition of these enzymes would reset the normal cellular acetylation levels. So far, HDAC inhibitors are the most well studied and efficient epidrugs. They can be categorized in hydroxamic acids, benzamides, short-chain fatty acids and cyclic peptides according to their chemical properties and their mechanism of action (89)Two pan-HDACis have been already approved for clinical use by the US Food and Drug Administration (FDA). Namely, Vorinostat and Romidepsin are effectively used for the treatment of cutaneous T-cell lymphoma (9). Supplementary clinical studies examine HDACis application for treating other cancer types.

HAT inhibitors (HATi) are also in development, but the structural differences among the families makes their design difficult. Curcumin, anacardic acid and garcinol are natural compounds that target mainly the p300/CBP activity (51). In addition, synthetic HAT inhibitors are extensively studied and tested (90). However, HAT inhibitors present low permeability and specificity, which explains their limited application in cancer treatment. Therefore, ongoing research focuses on their optimization (specificity, efficacy, toxicity).

Unlike HDAC and HAT inhibitors, the use of HDAC and HAT activators in epigenetic cancer therapy is not so widespread yet. To date, HAT activators are under investigation and the best described is CTBP which derives from anacardic acid and stimulates p300 *in vitro*. Likewise, HDAC activators are not well studied but they are promising tools for epigenetic cancer therapies (51).

Currently, the development of small molecules against the acety-lysine- reading bromodomain protein module is gaining significant interest (91). A number of inhibitors targeting the bromodomains have been designed and two of them, JQ1 and I-BET151, present efficient antitumoural activity in a series of hematopoietic malignant cell lines and in animal models. JQ1 shows high affinity for the first BRD4 bromodomain and when tested in NUT midline carcinoma cell lines, it induces rapid differentiation and growth arrest. Moreover, murine xenograft NMC models treated with JQ1 presented

tumor differentiation and increased survival (92). JQ1, as well as I-BET151, had beneficial effects in MLL-translocated leukemias both *in vitro* and *in vivo* (75,83).

What is interesting and promising about bromodomain ligands is that they might be used as a therapeutic strategy to target oncoproteins that are undruggable. For instance, the development of an inhibitor against MYC has been challenging over 30 years. However, as aforementioned it is likely that BET inhibitors exert at least a part of their anticancer activity by regulating Myc expression since MYC downregulation is a frequent observation after BET inhibition (84).

It is possible that many of the epidrugs act synergistically. So the simultaneous therapy with different epidrugs or with epidrugs combined with conventional chemotherapies may increase the therapeutic efficacy and decrease the possibility of drug resistance. Moreover, a new group of epidrugs [epigenetic multiple ligands (epi-MLs)] is introduced. Epi-MLs are synthetic compounds carrying chemical groups that target more than one class of epigenetic modifiers suggesting higher efficacy (51).

Overall, it seems that all the epigenetic players that modulate histone lysine acetylation are candidates for epigenetic based therapy of cancer. Despite the successful results that small molecules and natural compounds show in pre-clinical and clinical trials there is a number of questions that has to be addressed about epidrugs in general. One of them concerns selectivity. Epigenetic modifiers are ubiquitously expressed and they have also non-histone substrates. Consequently, they might have pleiotropic effects. The issue of safety and tolerance of epidrugs is also under investigation. The long-term safety of these agents has to be examined because modulation of the epigenetic "landscape" can reprogramme all cells. Therefore, side effects on stem or germ cells may be observed over time (9). The reason that epidrugs are effective against hematological malignancies and not against the majority of solid tumors is still unknown. Finally, another point that needs further research is the reason why epigenetic inhibitors regulate only a few hundred and reproducible genes.

Conclusions and perspectives

Alterations in histone acetylation pathways affect mainly the transcription process. Distorted histone acetylation patterns are observed in several cancer types. Depending on the target genes, both reduced activity of histone lysine acetyltransferases and increased activity on the wrong targets contribute to the development and progression of oncogenic transformation. The non-histone targets of acetyltransferases including crucial oncoproteins and tumor suppressors are also affected by aberrant acetyltransferase activity leading to malignancies. Abnormal recruitment to mistargeted loci and/or upregulation of bromodomain containing proteins, the readers of histone lysine acetylation, results as well in tumurigenesis. Further investigation of histone acetylation profile in different cancer types and the regulation of acetylation writers, readers and erasers are needed to better appreciate the link of epigenetic deregulation and cancer. Having obtained this knowledge more efficient treatment approaches will be developed.

So far, epidrugs targeting all the players that modulate histone lysine acetylation have been designed and a number of them show significant therapeutic efficacy. Ongoing research in drug development focuses on optimizing the specificity, toxicity and tolerance of epidrugs as well as understanding their precise mechanism of action. Therefore, epigenetic therapy for specific cancer types is promising the upcoming years.

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