Molecular pathways: the complexity of the epigenome in cancer and recent clinical advances

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Running title: Cancer epigenome and therapeutic strategies

The authors declare that they have no conflict of interest.
Abstract

Human cancer is causally linked to genomic and epigenomic deregulations. Epigenetic abnormalities occurring within signaling pathways regulating proliferation, migration, growth, differentiation, transcription and death signals may be critical in the progression of malignancies. Consequently, identification of epigenetic marks and their bio-implications in tumors represents a crucial step towards defining new therapeutic strategies both in cancer treatment and prevention. Alterations of writers, readers and erasers in cancer may affect, for example, the methylation and acetylation state of huge areas of chromatin, suggesting that epi-based treatments may require “distinct” therapeutic strategies compared to “canonical” targeted treatments. Whereas anticancer treatments targeting HDACs (HDAC inhibitors) and DNA methylation have entered the clinic, additional chromatin modification enzymes have not yet been pharmacologically targeted for clinical use in patients. Thus, a greater insight into alterations occurring on chromatin modifiers and their impact in tumorigenesis represents a crucial advancement in exploiting epigenetic targeting in cancer prevention and treatment. Here, the interplay of the best-known epi-mutations and how their targeting might be optimized will be addressed.
Background

A number of epigenetic deregulations such as DNA methylation, histone modifications and miRNA-based modulation have been progressively reported as causally involved in tumorigenesis and progression. In the past decade, several chromatin-modulating enzymes have been discovered and classified, and their aberrations linked to cancer. The state of chromatin is widely controlled by specific DNA- and protein-related modifications. Understanding the histone code and its crucial role in biology led to the study of enzymes that “write” these modifications, the so-called writers, those that recognize modified chromatin, termed readers, and erasers, which are able to remove a specific modification (1). The interpretation of epigenetic changes underlines the phenotypic variability of cells belonging to the same genome. Therefore, identification of epigenetic marks and their bio-implications in tumors represents a crucial step towards defining new therapeutic strategies both in cancer treatment and prevention (2). Alterations of writers, readers and erasers in cancer may affect the status of chromatin in vast areas of the epigenome, thus suggesting that epigenetic-based treatments may require “distinct” therapeutic strategies compared to “canonical” targeted treatments (3).

Chromatin modifiers and cancer

Chromatin structure can be remodeled by covalent modifications of DNA involving methylation of cytosine within CpG islands and a multitude of histone modifications in both their N-terminal and C-terminal tails, or even in their globular domains. Histone modifications dynamically change chromatin structure (so-called “epigenetic plasticity”). Histone modifications are involved in the neutralization/deneutralization of the positive charges present on histones and alter chromatin structure by determining an on-off state of transcription. In particular, histone acetylation is performed by histone acetyltransferase enzymes (HAT), an example of writers. HATs acetylate conserved amino acids on histones through the transfer of an acetyl group from Acetil-CoA to form ε-N-acetyl-lysine, thus neutralizing positive cells. Histone acetylation is a post-translational
modification linked to transcriptional activation. Conversely, histone deacetylases (HDAC), an example of *erasers*, define the removal of acetyl groups from an $\varepsilon$-N-acetyl-lysine amino acid on a histone, thus preventing transcription by increasing positive charges of histone tails and so determining high-affinity binding with DNA. The fact that modulation by *writers* and *erasers* can also involve non-histone substrates adds a further level of complexity (4). Therefore, a great deal of attention has been focused on chromatin-modifying enzymes in order to define their deregulation in cancer and their potential as therapeutic targets.

**HATs and cancer**

HATs belong to one of two main categories. A-type HATs acetylate nucleosomal histones within chromatin, whereas B-type HATs play a much broader role in the cell. A-type HATs are classified into four families sharing sequence homology within the HAT domain and include: Gcn5/PCAF (5), MYST (6), p300/CBP (7) and Rtt109, the latter reported to be mycotic-specific (8). In prostate cancer both p300 and CBP are overexpressed, thus altering androgen receptor (AR)-responsive gene modulation. CBP is also involved in translocation t(8;16) in which MOZ, an acetyltransferase localized on 8p11, is fused to CBP (9). Mutations in p300 (more frequently C-terminal truncations) have been found in many tumors such as breast, pancreatic, colorectal, gastric, cervical and ovarian cancers (10-12). Recently, downregulation of PCAF has been correlated to gastric carcinoma progression linked to poor clinical outcome. Mutation in Tip60 is associated with the development of prostate cancer through deregulation of DNA repair and resistance to apoptosis. PCAF and Gcn5 are overexpressed in diseases of central nervous system in pediatric patients and Wilms tumors (13). Rtt109, correlated to modifications of histones H3 in K9, K27 and K56, plays a critical role in nucleosome assembly (Supplementary Table 1 and Figure 1).
Global (de)acetylation, HDACs and cancer

The balance between HDAC and HAT activities plays a crucial role in regulating gene expression. Future studies will be needed to verify whether the modulation of chromatin acetylation is causally linked to cancer development or if it is caused by complex epi-deregulations. Whether cancers characterized by global alterations of chromatin acetylation state may benefit (and how) from an epi-based therapeutic approach is an issue that still needs to be addressed further (Supplementary Table 2). HDACs play a key role in gene regulation and therefore in human cancers, such as leukemia (14). HDACs are divided into four classes: class I HDACs (HDAC1, 2, 3 and 8), which are homologous to Saccharomyces cerevisae Rpd3; class II (further divided into class IIa and IIb) HDACs (HDAC4, 5, 6, 7, 9 and 10), which are homologous to yeast Hda1; class III HDACs (sirtuins) including enzymes homologous to yeast Sir2 involved in modulation of longevity, metabolism and physiology; class IV comprising only HDAC11, which shares homology with class I and II. HDACs are overexpressed in several cancers. In particular, HDAC1 is highly expressed in many malignancies including gastric, colorectal, hepatic, breast and pancreatic cancer (15-17). HDAC2 has been found mutated in colon cancer and is overexpressed in esophageal, prostate and gastrointestinal carcinomas (18-20). Many other cancers associated with poor prognosis, such as prostate, gastric, colorectal cancer and chronic lymphocytic leukemia (CLL) (17, 19, 21, 22) have been reported to display HDAC3 overexpression. The class IIa histone deacetylase HDAC4 is expressed in a tissue-specific manner and promotes the growth of colon cancer cells through repression of the cell cycle regulator p21 (23). HDAC5 downregulation has been reported in lung cancer (24), while its overexpression has been found in colon cancer (25). Downregulated expression levels of HDAC6 have been observed in lymphoma (26), whereas higher expression levels are associated with oral squamous cell cancer (27). The role of HDAC8 has been investigated in CLL (chronic lymphoid leukemia) in children (28). Of the class III HDACs, SIRT1 is involved in carcinogenesis and above all in age-related neoplasms. In particular, sirtuins are correlated to aging, cancer and stress response. SIRT1 overexpression has been found in prostate, colon and skin
cancers as well as in acute myeloid leukemia (AML) (29). In addition to SIRT1, other sirtuins (such as SIRT4 and 7) are linked to cancer development (30). Conversely, low levels of SIRT2 have been observed in gastric carcinoma and in gliomas. For some sirtuins, such as SIRT3, the scenario is complicated by the fact that both upregulation and downregulation have been reported in different forms of breast cancer. SIRT4 loss may contribute to diabetes, a major risk factor for cancer. SIRT6, which also displays ADP-ribosyltransferase activity, is widely overexpressed in brain and skeletal muscle. Finally, SIRT7 promotes active transcription of rRNA genes and lower levels of this enzyme have been found in non-proliferating tissues such as heart, brain and skeletal muscle (Table 1).

**PRMTs, KMTs, KDMs and cancer**

Histones are methylated by enzymes including arginine methyltransferases (PRMTs) and lysine methyltransferases (KMTs). The presence or absence of methyl marks on specific histones is crucial for gene expression regulation and has implications in carcinogenesis. PRMT1 is an important component of mixed lineage leukemia (MLL) oncogenic fusion proteins (31). PRMT6 is responsible for H3R2 (histone H3 Arginine 2) methylation, counteracting H3K4me3 (histone H3 lysine 4 trimethylation) deposition. High levels of PRMT1 have been observed in breast and colon cancers (32). PRMT2 can interact with estrogen receptor (ER) and acts as a strong co-activator of androgen receptor (AR). PRMT3 is involved in the regulation of protein synthesis, while PRMT4, (CARM1, co-activator-associated arginine methyltransferase1), is known to control the arginine-regulated mechanism of transcription. CARM1 methylates histone H3 and the mutation in the presumed binding domain decreases methyltransferase and p160 co-activator regulation (33). CARM1 is overexpressed in breast tumors and is essential for estrogen-induced cell cycle progression (34). PRMT5 is highly expressed in a wide variety of lymphoma and leukemia cells as well as in gastric carcinoma and immortalized fibroblast cells (35).
Many KMTs are strongly associated with cancer. Lysine methylation occurs in a very large number of histones, deposited by KMTs and removed by lysine demethylases (KDMs). EZH2, an H3K27 KMT and polycomb repressive complex 2 (PRC2) component, is highly expressed in several solid tumors such as primary prostate cancer (36) and in pro-B cells (37). Overexpression of SUV39H1/2 (KMT1A/B) has been reported in dietary-induced tumors (38) and in colon cancer cells. The KMT G9a contributes to histone H3 lysine 9 (H3K9) dimethylation, involved in suppressor-gene silencing. G9a is overexpressed in various cancers such as leukemia, prostate and lung cancer as well as hepatocellular carcinoma (39). KMT1D (Eu-HMTase1) is overexpressed in gland tumors (40), while SETDB1/ESET co-operates with DNA methyltransferase in the silencing of promoter regions in tumors via trimethylation of H3K9. Numerous mutations and rearrangements of MLL1 (KMT2A) have been observed in leukemogenesis. MLL4 (KMT2D) is involved in liver oncogenesis in hepatitis B patients. In addition, misregulation of histone demethylases also causes or contributes to cancer. In breast carcinoma, the downregulation of KDM1 (LSD1) is correlated to the onset of metastasis. The aberrant regulation of jumonji domain demethylases has been found in various cancer cell lines. KDM2B (JHDM1B/FBXL11) abolishes the dimethylation state of histone H3 lysine 36 dimethylation (H3K36me2) or histone H3 lysine 4 trimethylation (H3K4me3) by causing the downregulation of several proteins involved in cell cycle such as p14, p15 and p16 in T-cell lymphomas. The KDM5 family, including RBP2 (KDM5A), PLU1 (KDM5B) and SMCX (KDM5C), are overexpressed in a wide variety of cancers such as gastric, cervical, lung, breast, prostate and kidney cancer as well as leukemias. Another family of lysine demethylases, KDM8 or JMJD5, targets H3K36me2. This enzyme is overexpressed in breast, thyroid, adrenal, bladder and liver cancers (41) and plays a key role in modulating cell proliferation (Figure 1 and Table 1).

**DNA methylation and cancer**

DNA methylation has frequently been described as a static silencing event. DNA methylation is not, however, static, as recently demonstrated by the variable 5mC-oxidation kinetics at distinct
genomic/functional loci and by the fact that 2HG (product of IDH-1 and -2) mutation has been found in leukemia (42).

In recent years genome-scale mapping of methylation has revealed that DNA methylation is involved in different (epi)-genetic settings. Methylation is catalyzed by DNA methyltransferases (DNMT). Several cancers are associated with quantitative or positional alterations of DNA methylation. Furthermore, DNMT3a mutations have been found in AML (43). Thus, DNA methylation is the subject of intense studies aimed at better understanding alterations involved in the transformation of normal cells into pro-cancerous cells (44) (45).

Clinical-Translational Advances

**HDAC inhibitors**

Much attention has been focused on HDACs inhibitors (HDACi), mainly because HDACs are often overexpressed in cancer. HDACi are therefore considered a potential strategy to reverse epigenetic aberrations associated with cancer and many compounds have been tested in clinical trials (46). For an in-depth description of the role of HDACi in cancer we refer the reader to (47), (48), (49) and references therein. Whether isotype-selective HDACi offer greater therapeutic benefits (or lower adverse effects) over broadly acting HDACi (pan HDACi) remains one of the issues to be addressed in the clinic (Supplementary Table 3 and Figure 1).

**HAT inhibitors**

Few and poorly validated small molecules modulating HATs are currently available. In the last decade, two substrates analogous to peptide-CoA conjugates, Lys-CoA and H3-CoA-20, have been identified as powerful p300 and PCAF inhibitors. However, their metabolic instability precludes their use as anticancer drugs. Other PCAF inhibitors include isothiazolones and analogs that act as specific acetylation inhibitors in a dose- and time-dependent manner, and their anticancer properties
have been studied in liver cancer cells. Polyisoprenylated benzophene derivates (PBDs) have been proposed as candidates for HAT modulation. Garcinol is a B-type PBD active against viruses, bacteria, gastric ulcers and cancer, such as colon adenocarcinoma (50). Garcinol derivates based on iso-garcinol (IG) have been synthesized. These include LTK-13, -14 and the disulphonyl-substituted derivate LTK-19. Another promising HAT inhibitor (HATi) is anacardic acid (AA), the main component of cashew nut shell liquid. This compound is able to reduce breast cancer cell proliferation by inhibiting ERα-DNA binding (51). Gcn5-specific inhibitors, Y-butyrolactones, have also been synthesized. Some studies suggest that Gcn5 inhibition mediated by these compounds occurs in vitro in several cancers such as leukemia, melanoma, ovarian, renal, prostate and breast cancers as well as in colon cancer cells (52). Curcumin, an inhibitor of p300, regulates tumor suppressor pathways and triggers mitochondrial-mediated death in tumors. Curcumin has been reported to be active in the prevention and treatment of kidney, lung, ovarian, cervical and liver cancers. The development of HATi is less advanced than that of HDACi, probably due to the fact that HATs are less overexpressed in cancer and more often mutated compared to HDACs. Nevertheless, the identification of selective HATi for mutated forms of HAT in cancer may offer a valid therapeutic strategy with expected features of tumor-specificity.

PRMT, KMT and KDM inhibitors

PRMT alterations correlate with many cancers. Hence, molecules able to modulate methyltransferase activity are desirable for cancer treatment. Chaetocin, a fungal mycotoxin, is a SUV39H1 methyltransferase inhibitor reported to exert antimielyoma activity in IL-6-dependent and -independent myeloma cell lines, and in vivo (53). BIX01294 was the first small-molecule inhibitor of G9a and GLP. BIX01294 has been reported to inhibit the histone methyltransferase EHMT2, which acts as a co-repressor for specific transcription factors and is strongly overexpressed in bladder carcinomas (54). A large virtual screening effort has been performed to identify PRMT inhibitors (PRMTi) using the ChemBridge compound collection containing more than 300,000
compounds. Among these, acyl derivatives of p-aminosulfonamides and dapsone have been selected. In particular, dapsone has been suggested for the treatment of glioblastoma (55). Intensive efforts are currently ongoing for the identification of novel selective PRMTi. Tranylcypromine (trans-2-phenylcyclopropylamine) and its analogs are among the best-known LSD1 (or KDM1) inhibitors. Tranylcypromine has been proposed for the treatment of sarcomas as well as fibrous and peripheral nerve sheath tumors (56). Furthermore, the therapeutic potential of these two inhibitors has been reported for the treatment of promyelocytic leukemia (57). The KMT inhibitor 3-Deazaneplanocin A (DZNep) interferes with the polycomb-repressive complex 2 and induces apoptosis in cancer cells such as in AML. Moreover, inhibition of EZH2 by DZNep reduces proliferation in breast cancer cell lines (58). In AML, co-treatment with DZNep and Panobinostat, an HDAC inhibitor, exerts an apoptotic effect on primary leukemia cells but not on normal cells (59).

**DNA methyltransferase inhibitors (DNMTi)**

The Food and Drug Administration (FDA) has approved the DNMTi 5-azacytidine (azacytidine) and 5-aza-2-deoxycytidine (decitabine) for myelodysplastic syndromes (MDS) (60). Decitabine has also been used to force re-expression of silenced estrogen receptor in triple-negative breast cancer. Another important DNMTi is zebularine, which is selectively incorporated into malignant cells but not into normal cells. Cancers such as ovarian and cervical carcinomas can be treated with the DNMTi hydralazine (61). Besides nucleoside DNMTi, non-nucleoside targeted molecules directly inhibiting DNMTs have been developed. Among these, the phosphorothioate antisense oligonucleotide MG98, currently in phase I study, has been tested in patients with high-risk myelodysplasia and AML (62). RG108 displays demethylating activity comparable to zebularine in lymphoid, myeloid and colorectal cancers. The quinoline-based compound, SGI-1027, inhibits DNMT1, DNMT3a and DNMT3b, and has been proposed for hepatocellular, cervical, prostate and breast cancer *in vitro* (63). Finally, the antibiotics mithramycin A and nanaomycin have recently been reported to inhibit DNMT3B re-activation (64).
**Targeting the complexity of epigenetic modifications**

Misregulation of the physiological pattern of chromatin modifications (mainly DNA methylation and histone modifications) can induce the promotion and progression of cancer. Chromatin modifications may be regulated by *writers* and *erasers*, and interpreted by *readers*. These events give rise to a complex epi-modulated scenario, where both single modifications/single enzymes and the interplay with other histone marks/enzymatic complexes (possibly mutually exclusive or additive) need to be taken into account in order to achieve a better understanding of epigenetic deregulation in malignancies. In recent years a large number of high-throughput screening (HTS)-based studies in cancer models have highlighted both the importance of specific histone marks and epi-enzymes in cancer and their interconnection with other marks in wide areas of chromatin, suggesting the importance of the positioning (potentially mutually exclusive) of marks and chromatin modifiers in cancer pathogenesis and progression. Such scenarios point to the existence of a very complex “code” of mechanisms and their deregulation in cancer, underlining the difficulties involved in evaluating these mechanisms as therapeutic targets. Additional complexity arises from the difficulty in determining a hierarchy in epigenetic mark deposition. The fact that new modifications are still being uncovered makes the combinatorial repertoire of epigenetic marks appear endless. Currently, detailed knowledge of a hierarchy – mutual exclusiveness or interdependence – is restricted to a handful of well-studied marks such as histone methylation on H3K4 or DNA methylation. Only in a few cases has a ‘true’ hierarchy been successfully established, such as the requirement of H2B ubiquitylation for the deposition of the active histone mark H3K4me3 (65). Mutual exclusiveness has been reported for repressive DNA methylation and histone H3 lysine 27 trimethylation (H3K27me3) on CpG-islands (66). A well-known example of hierarchy is the dependence of 5-hydroxymethylation of cytosines on 5-methylcytosine as its substrate.

Translating our current knowledge from bench to bedside will involve identifying and developing new forms of “targeted” treatment, where large areas of chromatin within cancer cells are likely to
be influenced by the forced modification of their epigenetic status. This consideration should lead to a re-evaluation of the therapeutic scheme for existing epi-based cancer treatments as well as to a cautious patient stratification currently complicated by the presence of very few, if any, epi-biomarkers of response to treatment and prognosis. For example, only the predictive power of HR23B for clinical response to HDAC inhibitors in CTCL seems to be confirmed (67). Whether within this complex framework the anticancer action of epi-based modulators can be linked to ‘pure’ epi-effects alone and/or to what extent non-epigenetic action needs to be evaluated remains unclear. Furthermore, the fact that apparently opposite deregulations of the same epi-target have been reported complicates the interpretation of cancer epigenome. These contradictory findings might be explained by the hypothesis that opposite quantitative epi-alterations may lead, in some cases, to similar readouts in cancer, causing or contributing to a complex disorder of chromatin (and related gene expression) and its potential combinatorial patterns. As things stand, further knowledge is needed to mine the real value of some of the epi-target deregulations in cancer as well as to identify differences and specificities both for cancer disease and epi-modifiers. An additional and largely unexplored level of complexity is presented by the modulation of non-histone targets by small epi-molecules and by the fact that very few epi-based treatments (mainly HDACi and DNA demethylating agents) have actually entered the clinic. Lessons learned from the use of HDACi in cancer treatment suggest, for instance, that modulation of histone acetylation should be considered as a readout of the effects of treatment and not, as initially proposed, as a parameter of clinical response to treatment. Thus, one parameter of choice might be represented by the quantitatively unbalanced presence of writers/readers/erasers (overexpression or silencing) or by a specific mutation in one of these enzymes. However, while the selection of patients based on HDAC expression levels might prove useful, at least in in vitro settings, this parameter does not always seem to be predictive of a better response. The scenario might, however, be different when epi-enzymes are mutated in cancer. This type of epigenetic modification is currently under investigation to identify and validate biomarkers, and may allow patient stratification. It also offers the exciting
possibility of synthesizing selective small molecules able to modulate only the mutated enzyme, thus acquiring features of tumor-selective action. Interestingly, different categories of enzymes appear to act differently: HDACs, for example, are more often quantitatively modulated in cancer (with the exception of HDAC2 mutation), whereas HATs seem more frequently mutated. However, the implications of this difference still need to be investigated. Moreover, a further level of complexity is added by new discoveries continuously being made in this field: novel chromatin marks are identified and the efforts for mining these targets (alone and within the context of others) may rapidly change our view. For example, hydroxymethyl cytosine and its modulation is at present the focus of discussions aimed at understanding its mode of action and its potential role in cancer. Finally, it is debated whether more selective or broader acting chromatin modulators should be chosen as an approach to epi-treatment in cancer. While mutated targets might benefit from a selective epi-drug approach (better if active exclusively on the mutant), a broad modulator might present greater advantages in the case of concomitant alterations of different chromatin modifiers and marks. Multiple epi-modulators (targeting more than one class of enzymes, for instance) may also represent a promising approach (68), as could the creation of hybrid molecules able to act broadly on a class of epi-modifiers and to simultaneously mark a specific non-epi-target within cancer cells. This last approach, which still needs to be fully validated, might “re-modulate” chromatin in a more targeted manner.

**Acknowledgements**

This work was supported by EU: Blueprint (contract no. 282510), ATLAS (contract no. 221952); Epigenomics Flagship Project EPIGEN (MIUR-CNR); the Italian Association for Cancer Research (AIRC no. 11812); Italian Ministry of University and Research (PRIN_2009PX2T2E_004); PON002782; PON0101227. We apologize to authors whose work could not be cited due to restrictions in the number of references. We thank C. Fisher for linguistic editing of the manuscript. The authors declare that they have no conflict of interest.
Figure and Table Legends

Figure 1. Schematic representation of histone acetylation and methylation: chromatin marks and epi-enzyme deregulation in cancer. For histone methylation: **SUV39H1/2**: suppressor of variegation 3-9 (Drosophila) homolog 1/2; **G9a** or **EHMT2**: euchromatic histone-lysine N-methyltransferase 2; **SETDB1**: SET domain bifurcated 1; **MLL1/2**: lysine N-methyltransferase 2; **Ash1**: absent, small, or homeotic discs 1; **Set1**: spin echo T1 sequences; **Smyd3**: SET domain-containing protein with histone methyltransferase activity on histone H3 K4; **PRMT6**: protein arginine N-methyltransferase 6; **PRTMT1**: protein arginine N-methyltransferase 1; **SUZ12**: suppressor of zeste 12 protein homolog; **EED-EZH2**: enhancer of zeste homolog 2; **Set2**: spin echo T2 sequences. For histone acetylation: **Gcn5**: general control of amino-acid synthesis, yeast, homolog-like 2; **CBP**: CREB binding protein; **p300**: protein 300; **PCAF**: p300/CBP-associated factor; **MOZ**: monocytic leukemia zinc finger protein; **MYST3**: MYST histone acetyltransferase (monocytic leukemia) 3; **MORF**: monocytic leukemia zinc finger protein; **MYST4**: MYST histone acetyltransferase (monocytic leukemia) 4; **Tip60**: HIV1 Tat interacting protein; **Rtt109**: regulator of Ty1 transposition protein 109. For histone demethylation: **KDM1**: lysine-specific demethylase 1 (LSD1); **KDM2B**: lysine-specific demethylase 2B; **JHD**: JmjC domain-containing histone demethylation protein; **M1B**: methylation-inhibited binding protein 1; **KDM8**: jumonji domain of human Lysine-specific demethylase 8; **JMJD5**: jumonji domain-containing protein 5; **SET8**: SET domain-containing protein 8; **SUV4**: suppressor of variegation 4 homolog 2. For histone deacetylation: **HDAC1**: histone deacetylase 1; **HDAC2**: histone deacetylase 2; **HDAC3**: histone deacetylase 3; **SIRT1**: silent information regulator 1. Chromatin enzymes able to deposit or erase an epigenetic mark are indicated (in color code) as *writers* and *erasers*, respectively. Epi-drugs able to inhibit the activity of chromatin enzymes are indicated as *blockers*.

Table 1. HDACs, SIRTs, HMT & KDM in cancer. Main alterations of HDACs, SIRTs, HMT & KDM in cancer are listed.
Bibliography


Yildirim E, Zhang Z, Uz T, Chen CQ, Manev R, Manev H. Valproate administration to mice increases histone acetylation and 5-lipoxygenase content in the hippocampus. Neuroscience letters. 2003;345:141-3.


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<th>FUNCTIONS AND ALTERATIONS IN CANCER</th>
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<tr>
<td>HDAC1</td>
<td>Downregulated in CRC primary tumors; upregulated in breast, prostate, gastric and hepatic cancers</td>
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<td>HDAC2</td>
<td>Truncating mutation in colon, gastric and endometrial cancers; Overexpressed in prostate and gastrointestinal cancers</td>
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<td>HDAC3</td>
<td>Overexpressed in chronic lymphocytic leukemia</td>
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<td>HDAC8</td>
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<tr>
<td>HDAC4</td>
<td>Downregulated in lung carcinoma and overexpressed in colon, prostate and breast cancers</td>
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<td>HDAC5</td>
<td>Downregulated in lung cancer and upregulated in colon diseases</td>
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<td>HDAC7</td>
<td>High expression in childhood acute lymphoblastic leukemia (ALL) and colorectal cancer</td>
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<td>HDAC9</td>
<td>Overexpressed in medullo-blastoma</td>
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<td>HDAC6</td>
<td>Downregulated in lymphoma; high expression in oral squamous cancer</td>
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<td>SIRT1</td>
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<td>SIRT2</td>
<td>Reduced expression in human brain tumoral cells</td>
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<td>SIRT3</td>
<td>Is a promoter of cell proliferation and survival in oral cancer carcinogenesis</td>
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<td>SIRT6</td>
<td>Its loss contributes to the accumulation of mutations</td>
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<td>SIRT7</td>
<td>Increased expression in breast cancer; low levels in heart, brain and skeletal muscle</td>
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<td>HDAC11</td>
<td>Regulates OX40 ligand expression in Hodgkin lymphoma</td>
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<td>Is associated to Er- and is up-regulated in breast cancer</td>
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<td>PRMT4 (CARM1)</td>
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Table 1: HDACs, SIRTs, HMT & KDM in cancer.
Histone methylation

- SUV39H1/2, G9a, SETDB1
- ML1/2, Ash1, Set1, Smyd3
- PRMT6, PRMT1

Histone acetylation

- Gcn5, CBP, p300, PCAF, MOZ/MYST3, MORF/MYST4, Tip60, Rtt109

K5

- K4
- R2, R3

K9

- K27
- K36

Set2

K36

H1

H2A

H2B

H3

H4

KDM1/LSD1

KDM2B/JHD M1B

KDM8/JMJD5

SET8/SUV4

Histone demethylation

Histone acetylation

KDM inhibitors

HDAC inhibitors

Writers

Erasers

Blockers

KDM inhibitors

HDAC inhibitors

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Clin Cancer Res Published OnlineFirst August 17, 2012.