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Epigenetic Drugs in Cancer Therapy- A Review

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ABSTRACT

Cancer is a disease that results from the successive accumulation of genetic and epigenetic alterations. As epigenetic alterations are reversible, they offer great promise for treatment of cancer. Cancer is an epigenetic disease at the same level that it can be considered a genetic disease. These epigenetic changes may result from environmental factors and may lead to chromosomal instability, activation of endogenous parasitic sequences, loss of imprinting, illegitimate expression, aneuploidy, and mutations, and may contribute to the transcriptional silencing of tumor suppressor genes all of which increase the risk of cancer. CpG island methylation can also be used as a biomarker of malignant cells and may constitute a good target for future therapies.

INTRODUCTION

Epigenetics refers to heritable changes in gene expression that occur without alteration in DNA sequence. Epigenetics is the study of heritable changes in gene expression that does not involve changes to the underlying DNA sequence — a change in **phenotype** without a change in **genotype** which effects how cells read the genes. These epigenetic changes may result from environmental factors or may even occur due to DNA damage which is very frequent and they turn the genes "on" and "off" affecting how the cells read out the genes.

MECHANISM

Mechanisms that produce such changes are mainly DNA methylation and histone modification, each of which alters how genes are expressed without altering the underlying sequence. Epigenetic mechanisms can be grouped into 3 categories as:

1. DNA methylation
2. Histone modifications and
3. Nucleosome positioning.

DNA methylation

The addition of a methyl- group to one of the bases in the deoxyribonucleic acid (DNA) chain is called DNA Methylation, does not change the primary DNA sequence and it is therefore considered to be an epigenetic modification. DNA methylation is repressive to transcription, and therefore constituting an important mechanism for gene silencing in embryonic development and inactivation of defined tumor suppressor genes in human cancers. DNA methylation is involved in the regulation of several cellular processes including chromatin stability, imprinting, X chromosome inactivation and carcinogenesis.

In mammals, DNA methylation occurs mainly on the fifth carbon of the cytosine base, forming 5-methylcytosine or 5-methylcytidine (5-mC), and it is almost exclusively found at CpG dinucleotides. 5-mC is a potent epigenetic marker and regulator of gene expression. Methylated CpG clusters – named CpG islands, at gene promoters have been associated with gene inactivation. DNA methylation is catalyzed by a family of enzymes called DNA methyltransferase and includes DNMT1, DNMT3a and DNMT3b. DNMT3a and DNMT3b are known as de novo methyltransferases and which methylate previously unmethylated CpG

dinucleotides. On the other hand, DNMT1 is known as a maintenance methyltransferase, and it methylates hemimethylated DNA during replication.

The reverse reaction that is DNA demethylation is believed to involve the successive oxidation of 5-mC to 5-hydroxymethyl- (5-hmC), 5-formyl- (5-fC), and 5-carboxy- (5-caC) cytosine in a process that involves the Tet family of enzymes.

Chromatin Modifications

Chromatin is comprised of histones and DNA forming nucleosomes. A 147bp of DNA chain wrapped around the 8 core histone forms the basic chromatin unit, the nucleosome. The primary functions of chromatin are to package DNA into a smaller volume to fit in the cell, to strengthen the DNA to allow mitosis and meiosis and prevent chromosome breakage, and to control gene expression and DNA replication. In mammals, chromatin is mainly found as a condensed transcriptionally silent form called heterochromatin, which constitutes telomeres, pericentric regions and areas rich in repetitive sequences. Euchromatin is instead less condensed, and it contains most actively transcribed genes.

A vast number of proteins participate in shaping chromatin structure, including histones and other chromatin interacting proteins such as transcription factors and DNA repair proteins. Chromatin remodeling complexes have the ability to change the chromatin architecture by modulating the interaction between nucleosomes and DNA, which is often achieved by adding post-translational modifications to histones, serving as an epigenetic mechanism.

Histone modifications

Each nucleosome contains two subunits each of histones H2A, H2B, H3 and H4, known as the core histones. The linker histone H1 does not form part of the nucleosome itself but seems to act as stabilizer of the internucleosomal DNA. Post translational modifications of histones serve to allocate the genome into “active” regions or euchromatin where DNA is accessible for transcription, and “inactive” regions or heterochromatin where DNA is more compact and therefore less accessible for transcription thus altering how cells read the genes. Widely studied histone modifications are as follows:

Histone acetylation

Acetylation on histone tail (lysine residues) leads to relaxation of the chromatin structure and allows the binding of transcription factors and significantly increases gene expression. The enzymes responsible for regulating the acetylation of histone tails are histone acetyltransferases (HAT) and deacetylases (HDAC). It is a reversible process.

Histone methylation

Histone methylation can either repress or activate transcription depending on location. Arginine methylation of histone H3 and H4 promotes transcriptional activation, whereas lysine methylation of histone H3 and H4 is implicated in both transcriptional activation and repression, depending on the methylation site. Lysine residues can be methylated in the form of mono-, di-, or tri-methylation, providing further functional diversity to each site of lysine methylation. Histone methylation is a reversible process.

There are two types of histone methyltransferases:

1. Lysine-specific histone methyltransferases and
2. Arginine-specific histone methyltransferases.

Histone demethylases are involved in the reverse process of histone demethylation and can also be classified based on the residue they modify as:

1. KDM1/LSD1 (lysine-specific demethylase 1) is involved in demethylation of mono- and dimethylated lysines and
2. JmjC (Jumonji domain-containing) histone demethylases are able to demethylate mono-, di-, or tri-methylated lysines.
3. PAD4/PADI4 is involved in removal of arginine methylation and methylated arginine is converted into citrulline.

Histone Phosphorylation

All nucleosome core histones are phosphorylated and this modification is critical as intermediate step in chromosome condensation during cell division transcriptional regulation and DNA damage repair. Histone phosphorylation seems to function by establishing

interactions between other histone modifications, serving as platform for effector proteins, leading to a downstream cascade of events.

The markers for mitosis are phosphorylation of histone H3 at S10, involved in chromatin compaction and phospho-T120 in histone H2A, linked to regulation of chromatin structure and function during mitosis. Whereas, phosphorylation of H2AX at S139 (resulting in γH2AX), has been identified as one of the earliest event occurring after DNA double-strand break and serves as recruiting point for DNA damage repair proteins. Histone H2B phosphorylation hasn't been studied as well as H3 and H2A phosphorylation, but recent findings suggest that this modification facilitates apoptosis-related chromatin condensation, DNA fragmentation and cell death.

Histone Ubiquitylation

Histone H2A and H2B are the most abundant ubiquitylated proteins found in the nucleus, although ubiquitylated histone H3 and H4 have also been described. The most abundant forms of ubiquitylated histones are monoubiquitylated H2A on K119 and monoubiquitylated H2B on K123 (yeast)/K120 (vertebrates). Polyubiquitylated histones have also been described, such as K63-linked polyubiquitylation of H2A and H2AX. Monoubiquitylation of H2A is catalyzed by Polycomb group proteins, and it is mostly associated with gene silencing. The main enzyme responsible for monoubiquitylated H2B is Bre1 in yeast and its homologs RNF20/RNF40 in mammals. Monoubiquitylated H2B is mainly associated with transcription activation. Like other histone modifications, monoubiquitylation of H2A and H2B is reversible, and it is tightly regulated by histone ubiquitin ligases and deubiquitylating enzymes.

NON-CODING RNAs

About 90% of the eukaryotic genome is transcribed and only 1 – 2% of these transcripts encode for proteins. The majority are transcribed as non-coding RNAs (ncRNAs). ncRNAs play a big part in epigenetic regulation of gene expression in addition to their roles at the transcriptional and post-transcriptional level.

Non-coding RNAs can be divided into two main types:

a. Infrastructural and

b. Regulatory ncRNAs.

Infrastructural ncRNAs seem to have a housekeeping role in translation and splicing and include species such as ribosomal, transfer, small nuclear RNAs.

Regulatory ncRNAs are more interesting from an epigenetic point of view as they are involved in the modification of other RNAs. They can be further classified into:

MicroRNAs (miRNAs)

MiRNAs are small single-stranded molecules containing 20 – 24 nt which are derived from transcripts forming distinctive hairpin structures called pre-miRNA. The hairpin is processed into mature miRNA and forms the RNA-induced silencing complex (RISC), which contains miRNA-interacting proteins such as Dicer. The miRNAs pair with complementary sequences on target mRNAs transcripts through the 3'UTR, leading to gene silencing of the target.

Piwi-interacting RNAs (piRNAs)

piRNAs are small ncRNA containing 24 – 31 nt that are able to form complexes with Piwi proteins of the Argonaute family. pizRNAs are characterized by a uridine at the 5'end and a 2'-O-methyl modification at the 3' end. They play a key role in the silencing of transposable elements during germ line development.

Small interfering RNAs (siRNAs)

siRNAs are long linear dsRNA processed by Dicer into mature 20 – 24 nt siRNAs that direct silencing when loaded onto RNA-induced silencing complex. They mediate post-transcriptional silencing by a process called RNA interference (RNAi), where they interfere with the expression of a complementary nucleotide sequence.

Long non-coding RNAa (lncRNAs)

lncRNAs are considered as non-protein coding transcripts which are > 200 nt in length. Many of the lncRNAs are subject to splicing, polyadenylation, and other post-transcriptional modifications.

A subgroup of lncRNAs, named large intergenic non-coding RNAs (lincRNAs) are marked by trimethylation of K4 on histone H3 (H3K4me3) at their promoter and trimethylation of

K36 on histone H3 (H3K36me3) along the transcribed region. LincRNAs are involved in epigenetic gene silencing, and in tumor development by promoting expression of genes involved in metastasis and angiogenesis.

Enhancer RNAs (eRNAs)

eRNAs are non-coding transcripts with an average of 800 nt, and they are produced from regions enriched with monomethylated lysine 4 on H3, RNA Pol II and coactivators such as P300, which differentiates them from lncRNAs. It has been postulated that eRNAs function as transcriptional activators.

Promoter-associated RNAs (PARs)

PARs are non-coding transcripts that range from 16–36 nt to 200 nt, and they are generally expressed near the TSS (Transcription start site) or in upstream elements of the promoter. Most of the PARs are associated with highly expressed genes, but they are weakly expressed and with short half-lives. PARs are also connected with transcriptional activation.

CANCER EPIGENETICS

Cancer is a disease that results from the successive accumulation of genetic and epigenetic alterations. There is a number of *in-vivo* models of specific pathways of carcinogenesis that are very useful for the characterization of epigenetic mechanisms that link environmental exposures or genetic susceptibility and cancer progression. Because epigenetic alterations are thought to be reversible, they offer great promise for treatment of cancer.

DNA Methylation

The hypermethylation of the CpG islands of certain promoters is frequently observed. Global hypomethylation occurs mainly at repetitive sequences, promoting chromosomal instability, translocations, gene disruption and reactivation of endoparasitic sequences. LINE family member L1, are hypomethylated in a wide range of cancers, including breast, lung, bladder and liver tumors. Hypomethylation at specific promoters can activate the aberrant expression of oncogenes and induces loss of imprinting (LOI) in some loci. MASPIN (also known as SERPINB5), a tumor suppressor gene becomes hypermethylated in breast and prostate epithelial cells and appears to be hypomethylated in other tumor types. MASPIN hypomethylation, and therefore its expression, increases with the degree of dedifferentiation

of some types of cancer cells. Other examples of hypomethylated genes in cancer include S100P in pancreatic cancer, SNCG in breast and ovarian cancers and melanoma-associated gene (MAGE) and dipeptidyl peptidase 6 (DPP6) in melanomas. Whereas, hypermethylation is observed at specific CpG islands. The transcriptional inactivation caused by promoter hypermethylation affects genes involved in the main cellular pathways: DNA repair (hMLH1, MGMT, WRN, BRCA1), vitamin response (RARB2, CRBP1), Ras signaling (RASSFIA, NOREIA), cell cycle control (p16INK4a, p15INK4b, RB), p53 network (p14ARF, p73 (also known as TP73), HIC-1) and apoptosis (TMS1, DAPK1, WIF-1, SFRP1) etc.

HISTONE MODIFICATIONS

HDAC expression can be regulated by miRNAs, such as miR-449a, which, by repressing the expression of HDAC-1 in prostate cancer cells, regulates cell growth and viability. Several cancer types (e.g., colon, uterus, lung and leukemia) also bear translocations leading to the formation of aberrant fusion proteins, mutations or deletions in HATs and HAT-related genes, thus contributing to the global imbalance of histone acetylation. Besides the global loss of H4K16ac, cancer cells suffer a global loss of the active mark H3K4me3 and the repressive mark H4K20me3, and a gain in the repressive marks H3K9me and H3K27me3.

NUCLEOSOME POSITIONING

BRG1 and BRM, the ATPase subunits of SWI/SNF (chromatin remodeling) complexes, have been characterized as tumor suppressors and are silenced in about 15–20% of primary nonsmall-cell lung cancers. An oncogenic role for BRG1 as a p53 destabilizer has also been proposed. Mutations in SNF5, a subunit of the SWI/SNF remodeling complex, have been observed in sporadic renal rhabdoid tumors and in choroid plexus carcinomas, meduloblastomas and central primitive neuroectodermal tumors. Nucleosome remodeling is also involved in the transcriptional repression by promoter hypermethylation. Promoter hypermethylation results in the occupation of the TSS by a nucleosome, as has been reported for MLH1 in colon cancer.

EPIGENETIC DRUGS

Among the compounds that inhibit epigenetic processes, the most extensively studied are DNA methyltransferase inhibitors and HDAC inhibitors (HDACi). Here, we focus on

epigenetic modulators used in clinical trials (either completed or terminated) to treat human diseases.

HDAC INHIBITORS

Histone deacetylases are kenned to play a key role in the transcriptional machinery for regulating gene expression, to induce histone hyperacetylation and to affect gene expression. Consequently, they represent the target of therapeutic or prophylactic agents, HDACis, for diseases caused by eccentric gene expressions such as inflammatory disorders, diabetes, diabetic complications, homozygous thalassemia, fibrosis, cirrhosis, acute promyelocytic leukemia (APL), autoimmune diseases and tumors as well as organ transplant rejections and protozoal infections.

Acetylation and deacetylation of histones are carried out by histone acetyltransferases (HATs) and (histone deacetylases (HDAC) enzymes. The state of acetylation of histones is a consequential determinant of gene transcription. Deacetylation is generally associated with reduced transcription of genes whereas incremented acetylation of histones induced by the action of HDACi results in more preponderant transcription of genes.

A number of structurally diverse HDACi have been identified, many of which are or derive from natural products. These can be classified, according to their chemical structure, into the following categories:

(a)Hydroxamic acids and hydroxamic acid-based hybrid polar compounds.

(b)Cyclic tetrapeptides with the epoxyketone-containing amino acid (2S, 9S)-2-amino-8-oxo-9, 10-epoxy-decanoyl (Aoe).

(c) Cyclic tetrapeptides without Aoe and the depsipeptide FR-901228.

(d) short-chain and aromatic fatty acids.

(e) Benzamides and MGCD0103.

(f) Miscellaneous compounds.

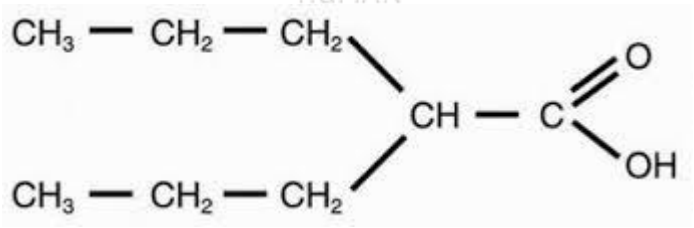
SHORT-CHAIN FATTY ACIDS

Their mechanism of action of short-chain adipose acids is predicated on the carboxylic group, occupying the acetate eluding tunnel that can have a zinc-binding function or compete with the acetate group relinquished in the deacetylation reaction. Two short-chain fatty acids, valproic acid (VPA) and sodium phenylbutyrate, are in clinical trials.

VPA, first used as an anticonvulsant and mood-stabilizing agent, is a pan-HDACi. Phase 1e2 clinical trials tested VPA alone or in combination treatment for lymphocytic leukemia, AML and myelodysplastic syndromes (MDS) in combination with 5-azacytidine, melanoma, HIV infection, autoimmune lymphoproliferative syndrome (ALPS), human T-lymphotropic virus type-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). There are also two clinical trials with sodium phenylbutyrate:

One in phase 2 for treatment of Huntington's disease and one in phase 1e2 for amyotrophic lateral sclerosis (ALS). Pivaloyloxymethyl butyrate is in phase 1e2.

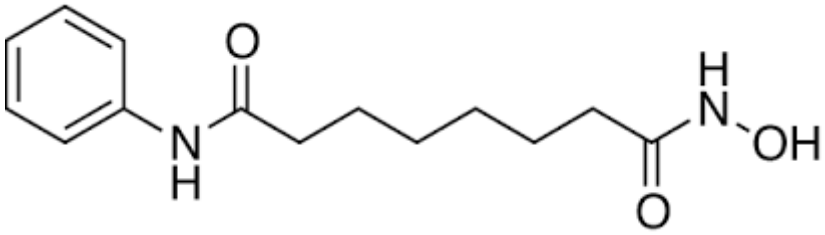
Clinical trials for chronic lymphocytic leukemia and lymphoma, malignant melanoma, and non-small cell lung carcinoma (NSCLC).



VALPROIC ACID: 2-propyl pentanoic acid

HYDROXAMIC ACIDS:

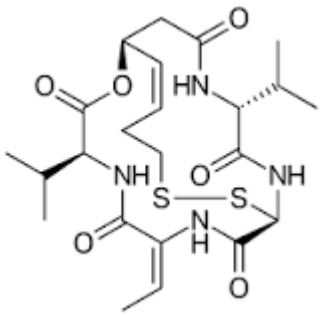
Hydroxamic acids include the majority of HDACi currently in clinical trials for the treatment of several human diseases such as cancer. The HDACi Vorinostat is at the most advanced stage in the clinical development. Vorinostat, a second-generation polar-planar compound, binds to the catalytic domain of histone deacetylases (HDACs).



VORINOSTAT: N-hydroxy-N'-phenyloctanediamide

BENZAMIDES: This class is composed of HDACi containing a characteristic 20-aminoanilide moiety able to contact concrete amino acids in the tube-like active site of the HDAC core, with or without coordination/chelation of zinc ion. These agents are in trials as single agents and in combination with other drugs. Clinical trials with MS-275, a class I selective inhibitor, include patients with a wide variety of hematologic and solid neoplasms such as leukemia, melanoma (MDS) and colorectal cancer. MGCD0103 is an isotype-selective HDACi that potently targets human HDAC1 but also exerts inhibitory activity against HDAC2, HDAC3, and HDAC11 *in vitro.* Some phase 1e2 clinical trials are for treatment of hematological diseases such as leukemia, lymphoma and also for solid cancers.

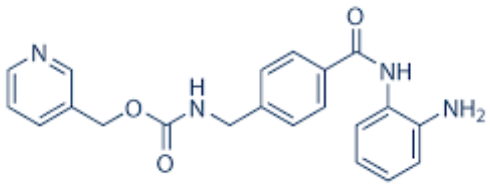
CYCLIC PEPTIDES: Romidepsin is a natural product obtained from the bacteria *Chromobacterium violaceum* acts as an HDACi. It was approved on November 5, 2009, by the FDA for the treatment of cutaneous T-cell lymphoma (CTCL). Terminated clinical trials show the activity of Romidepsin in treating lymphoma, MM, CTCL, MDS and solid tumors such as pancreatic, colorectal, lung, renal, bladder, brain, thyroid and ovarian cancers.



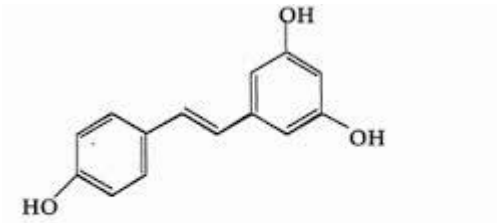
ROMIDEPSIN:Cyclo[(2Z)-2-amino-2-butenoyl-L-valyl-(3S,4E)-3-hydroxy-7-mercapto-4-heptenoyl-D-valyl-D-cysteinyl], cyclic (3→5)-disulfide

SIRTUINS: Sirtuins**,** are the silent information regulator 2 (Sir2) family of proteins. They are NADþ-dependent protein deacetylases, not modulated by HDACi. The deacetylase

activity of sirtuins is regulated by the cellular [NADþ]/[NADH] ratio; NADþ works as an activator, whereas nicotinamide and NADH inhibitors of sirtuins. Deacetylation and ADP-ribosylation are catalyzed by sirtuins where the cleavage of NADþ is the initial chemical step.

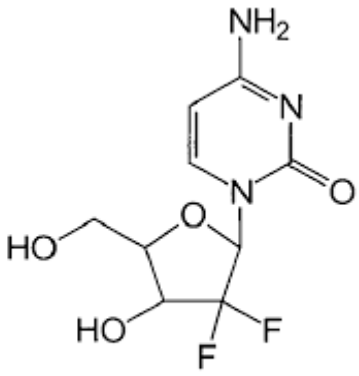


ENTINOSTAT



Resveratrol

(Trans-3,4',5-Trihydroxystilbene; MW 228.2)



Gemcitabine

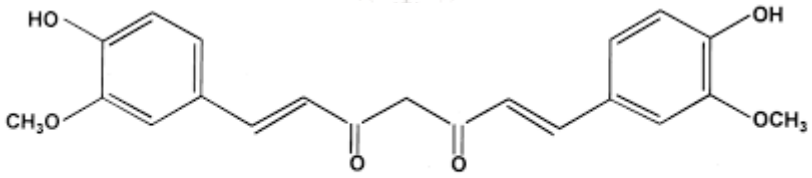
HAT INHIBITORS

The balance between the acetylation and deacetylation states of histones regulates transcription. Improper functioning of enzymes involved in these events is often associated with the manifestation of several diseases, including cancer, cardiac hypertrophy and asthma. Acetyltransferases (HATs) modulate gene expression by catalyzing targeted acetylation of the ε-amino group of lysine residues on histone and non-histone proteins. These enzymes are

therefore potential new targets for therapy. HATs can be classified into several families on the basis of number of highly conserved structural motifs which include

- The GNAT family (Gcn5-related N-acetyltransferase, eg: PCAF)
- The MYST group (MOZ, YBF2/SAS3 and TIP60) and
- The p300/CBP family. Although a wide number of transcriptional are now recognized to possess HAT activity, very few HAT inhibitors (HATi) have been identified to date.

Curcumin was identified as the first p300/CBP-specific cell permeable HATi. It does not affect the HAT activity of PCAF or histone deacetylase and methyltransferase activities. However, p300 HAT activity-dependent chromatin transcription is efficiently repressed by curcumin but not transcription from DNA template. Curcumin could also inhibit histone acetylation *in vivo.* It is the only HATi in clinical trials and exhibits great promise as a therapeutic agent. Its applications include atopic asthma, chronic obstructive pulmonary disease, multiple myeloma, irritable bowel syndrome, ulcerative colitis, Crohn's disease, breast cancer, Alzheimer's disease, pancreatic cancer, colorectal cancer, diabetes, and psoriasis.



CURCUMIN

CURCUMIN: (1E, 6E)-1,7-bis (4-hydroxy- 3-methoxyphenyl) -1,6- heptadiene-3,5-dione

HISTONE METHYLTRANSFERASES

On the basis of target residue for methylation, histone methyltransferases (HMTase) can be grouped into two different enzymatic classes:

1. Lysine methyltransferases and
2. Arginine methyltransferases.

Arginine methylation of histones H3 (Arg2, 17, 26) and H4 (Arg3) promotes transcriptional activation and is mediated by the family of protein arginine methyltransferases (PRMTs), including the co-activators PRMT1 and CARM1 (PRMT4). In contrast, a more diverse set of

histone lysine methyltransferases has been identified, all but one of which contain a conserved catalytic SET domain originally identified in the Drosophila Su[var]3e9, enhancer of zeste and Trithorax proteins. Lysine methylation has been implicated in both transcriptional activation (H3 Lys4, 36, 79) and silencing (H3 Lys9, 27, H4 Lys20).

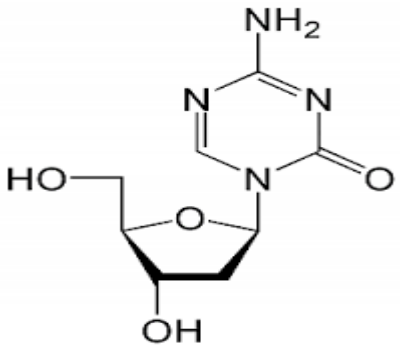
There are many well-known methylation sites on histones

HISTONE METHYLTRANSFERASE INHIBITORS

S-adenosylmethionine (SAM) and its analogs (e.g., SAH) were first applied inhibitors used as anti-cancer drugs. These compounds target not only HMTs but also other enzymatic classes using AdoMet as methyl-donor (such as DNMTs). Therefore, their use is limited by low specificity.

Chaetocin, a fungal mycotoxin and BIX-01294 (and its derivative BIX-01338, both hydrochloride hydrates) are used as inhibitors of G9a.Chaetocin is used G9a in low concentration as lysine methyltransferase inhibitor, without inhibition of other KMT enzymes (such as EZH2 or SET7/9). Inhibition mediated by chaetocin is competitive against the co-substrate SAM.

Other classes of drugs include DNA demethylase enzymes and their inhibitors, DNA methyltransferase enzymes and their inhibitors, Nucleoside analogs (eg. Decitabine), Small molecules (eg. Hydralazine, procainamide), natural molecules (eg., catechin, quercetin), antisense oligonucleotide inhibitors of DNMTs, non-coding RNAs, HMTLHDM inhibitors and HATLHDAC inhibitors.



DECITABINE: 5-Aza-2'-deoxycytidine

HYDRALAZINE: 1-Hydrazinophthalazine

CONCLUSION

Great progress has been made in the description of epigenetic modifications in normal and diseased tissues. Thus far, efforts in epigenetic research have mainly focused on cancer, but as the field has grown, it has provided new insights into other types of diseases, particularly neurological and autoimmune diseases. Epigenetic alterations are likely to be found in other disorders; indeed, they have already been described in cardiovascular diseases, metabolic diseases, myopathies and children born from assisted reproductive treatments.

The detailed study of the epigenetic maps would be of enormous use in basic and applied research and would be relevant for focusing pharmacological research on the most promising epigenetic targets. A key topic for future research is the implementation of mechanisms for the release of whole genome methylation and histone modification maps into public databases.

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