Research Focus

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Volume 1 • 2012

Shaping Epigenetics Discovery

Acetylation and Methylation: Epigenetic Modulators of Gene Expression

The dynamic structural and functional scaffold of chromatin plays a vital role in many cellular processes, and is influenced by both genetic and epigenetic factors. DNA methylation and the post-translational modifications of histones are regarded as the major epigenetic factors. These two processes work in concert to regulate gene expression.

Histone Acetylation

Histone acetylation and deacetylation play a major role in determining chromatin structure and function. In the deacetylated form, specific basic amino acids in histones are positively charged and interact with DNA's negatively charged phosphate groups and with negatively charged patches on neighboring nucleosomes. This promotes chromatin condensation to form heterochromatin. On the other hand, acetylation neutralizes these positive charges, promoting the less condensed and more accessible chromatin conformation known as euchromatin. Thus, histone acetylation, particularly of H3 and H4, has been linked to actively transcribed genomic regions, and histone acetylases (HATs) and histone deacetylases (HDACs) have been traditionally linked to transcriptional activation and repression, respectively.

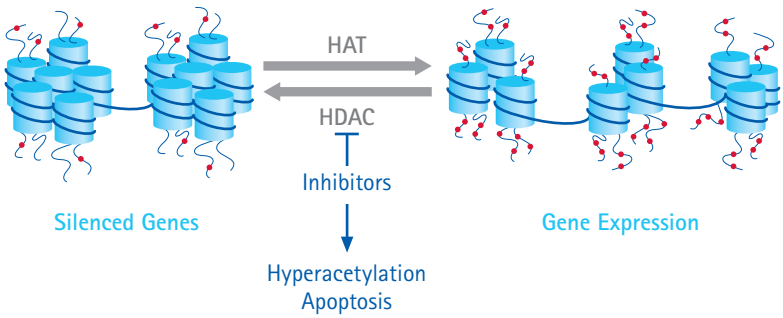
HATs catalyze the transfer of an acetyl group from acetyl-CoA to the ε-amino groups of specific lysines in histones, and are classified into several families based on sequence conservation. They include the GNAT (Gcn5-related N-acetyltransferase) superfamily, the p300/CBP family, the TFIIIC family, the MYST family (named after its founding members, MOZ, YBF2/SAS3, SAS2 and TIP60), TATA-box binding protein-associated factor TAFII-p250, and the nuclear receptor co-activators, e.g., ACTR, SRC-1, and TIF2.

HDACs catalyze the removal of the acetyl moiety from the ε-amino groups of lysines in histones. In mammals, eighteen different HDACs have been reported and are subdivided into four classes based on phylogenic analysis, sequence similarity to their founding yeast homologs, subcellular localization and enzymatic activities. Class I HDACs (HDAC 1, 2, 3, and 8) are widely expressed in tissues and are primarily located in the nucleus. Class II HDACs (HDAC 4, 5, 6, 7, 9, and 10) are much larger, display limited tissue distribution, and can shuttle between nucleus and cytoplasm. Class I and II HDACs are zinc-dependent enzymes. Class III HDACs (human SIRT 1 to 7) are a large family of sirtuins (silent information regulators or SIR),

with unique enzymatic mechanisms dependent on NAD^+; they do not contain a zinc-binding site. There is only one Class IV HDAC, HDAC 11. It localizes primarily to the nucleus, and its catalytic core shares sequence homology with those of class I and II HDACs. In humans, HDAC 11 is expressed mainly in brain, heart, muscle, testis, and kidney, and in several cancer cell lines.

Biological roles of acetylation

Histone acetylation and deacetylation have been implicated in many biological processes, such as cell differentiation and survival, double-strand DNA break repair, cell cycle progression, malignant transformation, cardiac function and remodeling, and plant acclimation to cold stress. Also, studies have linked the proliferative capacity of many solid tumors to the histone acetylation status. The loss of acetylation at Lys16 of H4 is a common characteristic of human cancer. Hence, HATs and HDACs have become some of the most promising targets in cancer therapy.



HDAC inhibitors are linked to increased apoptosis in cancer cells, in some cases via hyperacetylation (and, therefore, transcriptional activation) of proapoptotic genes.

Histone Methylation

Histone methylation is a more stable modification than acetylation and phosphorylation. In methylation reactions, the ε-amino group of certain lysine residues and the guanidinium group of certain arginine residues are methylated.

Lysine methylation

Except for yeast Dot1 and mammalian Dot1L, all known histone lysine methyltransferases (HKMTs; also known as protein lysine methyltransferases (PKMTs)) contain the conserved SET enzymatic domain (originally recognized as a conserved sequence in three *Drosophila* genes: *Su(var)*3-9; *En(zeste)*; and *Trx*). Dot1 and mDot1L contain novel enzymatic domains. Methylation of lysines 4, 36 and 79 of H3 is associated with active chromatin regions, while methylation of lysines 9 and 27 of H3 and lysine 20 of H4 is generally associated with silenced chromatin regions. Furthermore, lysine methylation can occur in monomethyl, dimethyl or trimethyl forms, which further expands its epigenetic information potential.

Arginine methylation

Arginine methylation is catalyzed by protein arginine methyltransferases (PRMTs). In mammals, it is typically seen on residues 2, 8, 17, and 26 of H3 and residue 3 of H4. Methylation at H3R17, H3R26 and H4R3 has been associated with gene activation, while methylation at H3R8 has been associated with gene repression. PRMT family members are classified as type I (PRMT1, 3, 4, 6, and 8) and type II (PRMT5 and 7); the enzymatic activity PRMT2 remains to be characterized. Type I PRMTs catalyze the formation of ω-N^G monomethylarginines (MMA) and ω-N^G, N^G-asymmetric dimethylarginines (aDMA); type II PRMTs catalyze the formation of MMA and ω-N^G, N '^{G}-symmetric dimethylarginines (sDMA).

Biological roles of histone methylation

Recent studies have implicated histone methylation in the maintenance of embryonic stem (ES) cells in the undifferentiated state, arginine demethylimination in transcriptional repression, histone lysine demethylases in transcriptional regulation, cancer cell proliferation and normal neuronal function, and the loss of trimethylation at Lys20 of H4 in human cancer. Studies have suggested that histone demethylation and deacetylation are tightly coupled.

DNA Methylation

DNA Methyltransferases

In mammalian cells, DNA methylation is catalyzed by DNA cytosine-5 methyltransferases (DNMTs), which transfer a methyl group from S-adenosyl-methionine to C-5 of cytosine. Five related mammalian DNMTs have been reported: DNMT1, 2, 3a, 3b, and 3L. DNMT1 is known as the maintenance methyltransferase, binds preferentially to hemimethylated DNA and plays a role in DNA mismatch repair. DNMT2 contains the conserved methyltransferase motif found in the other methyltransferases, but lacks the amino terminal regulatory domain, and has been shown to be an RNA methyltransferase. DNMTs 3a and 3b, known as *de novo* methylases, bind both unmethylated and hemimethylated CpG sites, and their activities may be dependent on other proteins. DNMTL (DNMT 3-like) lacks a catalytic domain and is devoid of DNA methyltransferase activity; it is believed to function as partner for the *de novo* DNMTs 3a and 3b, enhancing their catalytic activities.

Biological roles of DNA methylation

In the mammalian genome, about 70% of CpG dinucleotides are methylated. Many of the remaining nonmethylated CpGs are in CpG islands typically found in functional promoter regions. DNA methylation has long been viewed as an epigenetic marker of gene repression and plays important roles in heterochromatin formation, long-term silencing of repetitive elements, X-chromosome inactivation and in the establishment and maintenance of imprinted genes. However, more recent studies show that transcriptional activation is associated with cycles of DNA methylation and that DNMTs are involved in both addition and removal of methyl groups.

Additionally, differentiation of ES cells is shown to be associated with changes in DNA methylation that involve both demethylation and *de novo* methylation. While it is clear that DNA methylation plays an important role during development, and that the particular DNA methylation patterns at CpG islands are among the factors that underlie the production of various cell types in the body, there is still no consensus as to why development fails when DNA methylation is deficient.

Many research efforts have linked the DNA methylation status to neoplasia. Although early studies reported global genome hypomethylation in cancer, tumorigenesis is frequently associated with hypermethylation of CpG islands in promoters of tumor suppressor genes.

How does DNA methylation affect gene expression?

DNA methylation status can affect gene expression by various mechanisms. For example, DNA methylation could sterically hinder the binding of activating transcription factors to gene promoters, or could recruit repressor-type protein factors that specifically bind methylated DNA via their methyl-CpG-binding domains (MBDs); Methyl-CpG-binding proteins or DNMTs may bind/recruit HDACs or histone methyltransferases and thus influence histone modifications.

The diverse combinations of histone post-translational modifications and DNA methylation states lead to different functional consequences that affect both normal development and disease progression. Hence, the development of specific modulators of chromatin modifying enzymes and the detection of epigenetic alterations are crucial for studying the contribution of each of these epigenetic modifications in different biological processes.

NEW! EPIGENETICS RESEARCH TOOLS

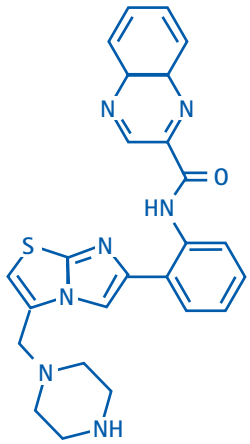
Small Molecules and Inhibitors

Product	Catalogue No.
HDAC Inhibitor XXIII, Tubastatin A	382187
HMTase Inhibitor V, UNC0224	382193
Protein Methyltransferase Inhibitor II, BIX-01338	539212
Histone Acetyltransferase p300 Inhibitor, C646	382113
HDAC Inhibitor XXII, NCH51	382185
LSD1 Inhibitor II, S2101	489477
Histone Lysine Methyltransferase Inhibitor	382190
Protein Arginine N-Methyltransferase Inhibitor, AMI-1	539209
JMJD2 Inhibitor, 5-carboxy-8HQ	420201
Ubiquitin E1 Inhibitor, PYR-41	662105

FEATURED PRODUCTS

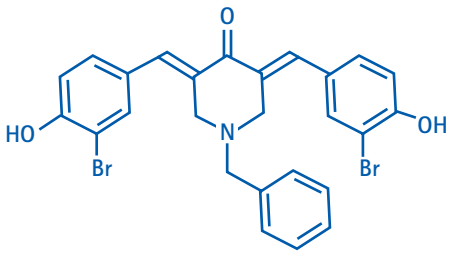
SRT1720

Sirtuins (class III HDACs) became the focus of intense research when it was discovered that their activation led to reduced incidence of aging and age-related diseases, including diabetes. SRT1720 is an orally bioavailable quinolinecarboxamide compound that acts as a potent, reversible allosteric inhibitor of SIRT3 activity.



CARM1 Inhibitor

CARM1 is an arginine-specific methyltransferase that plays roles in embryonic development and prostate cancer progression, presumably via chromatin remodeling. A cell-permeable (bis-benzylidene)piperidinone compound, CARM1 inhibitor is a potent, reversible and selective inhibitor of CARM1-mediated methylation over PRMT1 and SET7.

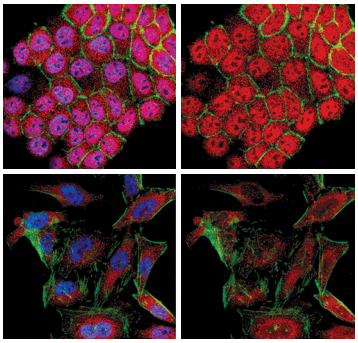


NEW! ANTIBODIES FOR EPIGENETICS RESEARCH

Anti-HDAC4

Catalogue No. ABE262

Histone deacetylases (HDACs) are recruited to specific promoters or chromosomal domains by association with DNA-binding proteins. HDAC4 does not bind DNA directly but interacts with the myocyte enhancer factors MEF2A, MEF2C, and MEF2D. This antibody recognizes HDAC4 at the C-terminus. Suitable for immunocytochemistry, immunoprecipitation, and Western blotting with human samples.



Confocal fluorescent analysis of A431and HeLa cells using a 1:500 dilution of Anti-HDAC4 (Red). Actin filaments have been labeled with Alexa Fluor® 488 dye-Phalloidin (Green). Nucleus is stained with DAPI (Blue). This antibody positively stains the nucleus and cytoplasm.

Additional Tools for Epigenetics Research

Description	Catalogue No.
Antibodies	
Anti-HDAC4	ABE262
Anti-Ars2	ABE280
Anti-ASF1a, clone MPH7	MABE90
Anti-Dimethyl-Histone H3 (Lys4)	07-030
Anti-Monomethyl-Histone H3 (Lys27)	07-448
Anti-LSD1	09-058
Anti-JMJD3	07-1533
Proteins & Enzymes	
HDAC1 Active recombinant protein	14-838
CpG MethylQuest™ Protein	14-921
Kits & Assays	
SIRTainty™ Class III HDAC Assay	17-10090
AbSurance™ Complete Core Histone Antibody Specificity Array	16-668
CpGenome™ Turbo Bisulfite Modification Kit	S7847
CpGenome™ Universal DNA Modification Kit	S7820
CpG MethylQuest™ DNA Isolation Kit	17-10035

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Lit No. TN1110ENEU BS-GEN-12-06988 Printed in the USA. 7/2012

References

Smith, Z.D., et al., 2012. Nature 484, 39.
Rahman, R., and Grundy, R. 2011. Int. J. Cancer 129, 2765.
Watanabe Y., and Maekawa M., 2010. Adv. Clin. Chem. 52, 145
Spannhoff, A., et al. 2009. Chem.Med.Chem. 4, 1568
Escargueil, A., et al. 2008. Mutat. Res. 658, 259.
Kangaspeska, S., et al. 2008. Nature 452, 112.
Latham, T., et al. 2008. Cell Tissue Res. 331, 31.
Liu, H., et al. 2008. J. Neurosci. Res. 86, 537.
Métivier, R., et al. 2008. Nature 452, 45.
Suzuki, M., and Bird, A. 2008. Nat. Rev. Gen. 9, 465.
Zheng, Y., et al. 2008. Med. Res. Rev. 28, 645.
Zhu, J., et al. 2008. Proc. Natl. Acad. Sci. USA 105, 4945.
Gilbert, N., et al. 2007. J. Cell Biol. 177, 401.
Guo, H-B., and Guo, H. 2007. Proc. Natl. Acad. Sci. USA 104, 8797.
Klose, R.J., and Zhang, Y. 2007. Nat. Rev. Mol. Cell Biol. 8, 307.
Ruthenburg, A.J., et al. 2007. Nat. Rev. Mol. Cell Biol. 8, 983.
Backs, J. and Olson, E. 2006. Circ. Res. 98, 15.
Bolden, J.E., et al. 2006. Nat. Rev. Drug Dis. 5, 769.
Minucci, S., and Pelicci, P. 2006. Nat. Rev. Cancer 6, 38.
Cheng, X., et al. 2005. Annu. Rev. Biophys. Biomol. Struct. 34, 267.
Cheung, P., and Lau, P. 2005. Mol. Endocrinol. 19, 563.
Fraga, M.F., et al. 2005. Nat. Genetics 37, 391.
Miao, F., and Natarajan, R. 2005. Mol. Cell. Biol. 25, 4650.
Villar-Garea, A., and Esteller, M. 2004. Int. J. Cancer 112, 171.
Shiota, K., et al. 2002. Genes Cells 7, 961.

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