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New Drugs

The promise and failures of epigenetic therapies for cancer treatment

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ABSTRACT

Genetic mutations and gross structural defects in the DNA sequence permanently alter genetic loci in ways that significantly disrupt gene function. In sharp contrast, genes modified by aberrant epigenetic modifications remain structurally intact and are subject to partial or complete reversal of modifications that restore the original (i.e. non-diseased) state. Such reversibility makes epigenetic modifications ideal targets for therapeutic intervention. The epigenome of cancer cells is extensively modified by specific hypermethylation of the promoters of tumor suppressor genes relative to the extensive hypomethylation of repetitive sequences, overall loss of acetylation, and loss of repressive marks at microsatellite/repeat regions. In this review, we discuss emerging therapies targeting specific epigenetic modifications or epigenetic modifying enzymes either alone or in combination with other treatment regimens. The limitations posed by cancer treatments elicit unintended epigenetic modifications that result in exacerbation of tumor progression are also discussed. Lastly, a brief discussion of the specificity restrictions posed by epigenetic therapies and ways to address such limitations is presented.

Introduction

The epigenome (epi meaning above or beyond) consists of heritable modifications to histones and DNA which are independent of changes to the linear DNA sequence.[1,2] These modifications are established by transiently or stably expressed proteins that respond to developmental cues, internal stimuli and environmental factors and include DNA methylation, histone acetylation, methylation, phosphorylation, ubiquitination, citrullination, sumoylation, and ADP ribosylation. Cues established during development allow cells with identical DNA to differentiate into a wide array of cell types, a process which coupled to environmental factors, can lead monozygote twins to establish different epigenetic patterns and consequently, different profiles of gene expression. Such alterations of cellular programming can dictate differences in susceptibility to disease and therefore, represent potential targets for pharmacological intervention.[3,4]

Epigenetic modifications are dynamically established by DNA methyltransfereases (DNMTs), histone acetyltranferases (HATs), histone methyltranferases (HMTs), kinases, and removed or modified by histone deacetylases (HDACs), histone demethylases (HDMs), ten eleven translocation protein 1–3 (TET1–3), and phosphatases in a highly regulated manner. Such modifications alter the conformation of chromatin by effecting covalent interactions or by acting as special docking sites for reader and effector proteins.[5] The interaction between chromatin modifying enzymes and reader/effector proteins regulates transcriptional activation or repression leading to varied cellular phenotypes. Epigenetic modifications are not stand alone processes, instead, are subject to considerable crosstalk among the different types of epigenetic marks. Epigenetic marks by themselves, or synergistically with other macromolecular modifications, function to either repress or activate transcription. For example, actively transcribing genes are enriched at the promoters with acetylation (AC), H3 trimethylation at lysine-4 (H3K4me3), and unmethylated CpGs. Further, enhancers are enriched with H3 methylation at lysine-1 (H3K4me1), and H3 acetylation at lysine-27 (H3K27AC), while gene bodies are enriched with H3 dimethylation at lysine-36 (H3K36me2), H3 acetylation at lysine-12 (H3K12AC) and DNA methylation (Fig. 1(top)).[6–9] On the other hand, the promoters of repressed genes are enriched with DNA methylation at CpG sites, H3 trimethylation at lysine-27 (H3K27me3), H3 trimethylation at lysine-9 (H3K9me3), which can also be present in enhancer and gene bodies, as well as overall loss of acetylation (Fig. 1(bottom)).[8,10–12] Unlike genetic mutations and gross structural defects that may permanently activate or inactivate genes, all epigenetic modifications identified to date are modifiable, thus conceivably allowing correction of aberrant epigenetic profiles (Fig. 1).

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Tumor cells undergo massive epigenetic reorganization, with the occurrence of oncogenic phenotypes associated with pronounced CpG-specific hypermethylation in the promoters of tumor suppressor genes, and generalized hypomethylation of the promoters of oncogenes, microsatellite regions and repetitive sequences.[13,14] Such changes in the epigenetic landscape contribute to both initiation of tumorigenesis and progression of oncogenic phenotypes. For example, in normal cells, the promoters of tumor suppressor genes are enriched with active transcription marks, such as H3K4me3, H3K9me, H4 acetylation at lysines-5,-8,-12,-16, and 20 (H4 -K5, -K8, -K12, -K16, and -K20 ACs), while satellite regions are enriched with repressive marks, such as H3K27me3, H3K9me2, and H4K16Ac (Fig. 2).[15,16] In tumor cells, the promoters of tumor suppressor genes lose nearly all acetylation, and acquire repressive marks including H3K27me3 and H3K9me (Fig. 2).[17] At repetitive/satellite regions, repressive marks such as DNA methylation (Me), H4K20me3, and H3K27me3 are lost leading to chromosome/microsatellite instability (Fig. 2).[18–20]

In recent years, therapies targeting epigenetic modifying enzymes (e.g. DNMTs, HATs, HDACs, kinases, HMTs, and HDMs), pathways and accessory proteins have been developed to either block or reverse the aberrant epigenetic modifications (Fig. 2). To date, four epigenetic drugs: 5-azacytidine (azacitidine or 5-aza-CR), 5-aza-2′-deoxycytidine (5-aza-CdR or decitabine), vorinostat (suberoylanilide hydroxamic acid (SAHA)), and romidepsin have been approved by the US Food and Drug Administration (FDA). Azacidine and decitabine were approved for the treatment of high risk myelodysplastic syndrome, while vorinostat and romidepsin were approved for cutaneous T cell lymphoma (CTCL). This review highlights some of the latest therapies targeting epigenetic enzymes, pathways and accessory proteins in cancer treatment.

Histone acetyltransferase (HATs) and HAT Inhibitors (HATis)

Histone acetylation involves the transfer of an acetyl group from acetyl coenzyme A (acetyl-CoA) to the ε-amino group of lysine by HAT.[21,22] HATs have been grouped into Types-A and -B HATs based on sequence divergence of the HAT domain and intracellular localization. This nomenclature, however, was confusing and a new nomenclature has been adopted as denoted in Table 1.[23] Type-A HATs are nuclear proteins that acetylate histones and other chromatin-associated proteins, while Type-B HATs are both nuclear and cytoplasmic and mainly acetylate *de novo* synthesized histones in the cytoplasm to promote their nuclear localization.[24,25] Type-A HATs consist of three families: GNATs, P300/CBP, and MYST. Thus far, histone acetyltransferase-1 (HAT1/KAT1) is the only Type B HAT shown to acetylate H3 at lysines-5 or 12 (K3K5/12).[22,26] The different HAT families show little sequence similarity with no homology domain, but most HATs contain a recognizable acetyl-CoA binding domain. Crystal structure analyses of all HATs have provided insight into how these enzymes interact with their substrates.[27] For example, X-ray crystallography of Type-A HATs uncovered a conserved core domain consisting of three-stranded β-sheets connected to long and parallel α-helices, and this core region supports the conserved interaction of the protein with the acetyl-CoA or related substrates.[27]

Despite conservation of this core domain, mutational, biochemical and enzymatic analyses showed that the different HAT families employ different mechanism to transfer the acetyl group. The GNAT family employs a one-step bi-bi ternary complex mechanism to transfer the acetyl-CoA. In this mechanism, acetyl-CoA and the substrate bind to form a ternary complex and the glutamate (Glu173 for KAT2A (GCN5) and Glu570 for KAT2B (hPCAF)) at the active site acts as a base and deprotonates the ε-amino group of lysine. This allows nucleophilic attack on the carbonyl carbon of

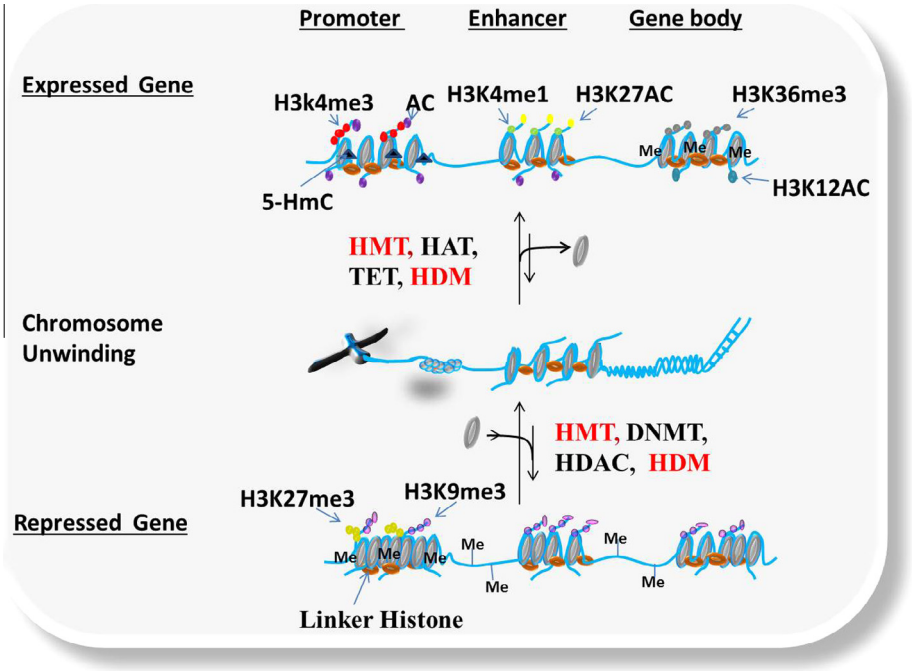

Fig. 1. Dynamic epigenetic regulation in normal cells showing crossed talk between epigenetic modifications resulting in transcriptional activation or repression. This figure depicts different epigenetic modifications under normal conditions for both expressed and repressed genetic targets. Histone acetyltransferases (HATs), histone methyltransferase (HMTs), ubiquitin ligases (E3), ten eleven translocation (TET) DNA hydroxylase (convert 5mC to 5HmC) and kinases add transcriptionally active epigenetic marks (e.g. AC, H3K4me3, H3K4me1, H3K27Ac, H3K36me3, H3K12Ac and 5HmC) to promoters, enhancers and gene bodies which promote the formation of euchromatin and transcriptional activation (top). DNA methyltransferease (DNMT), histone methyltransferase (HMT), ubiquitin ligase (E3), histone deacetyltransferase (HDAC) put repressive epigenetic marks (H3K27me3, H3K9me3, and DNA methylation) to promoters, enhancers and gene bodies promoting the formation of heterochromatin and transcriptional repression (bottom).