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Cracking the Code: The Promise of Epigenetics

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Completion of the human genome project together with continuing efforts to identify underlying genetic drivers of disease have led to tremendous advances in the discovery of novel therapeutics. In oncology, FDA approval last year of the kinase inhibitors vemurafenib and crizotinib, targeting the mutant BRAF V600E and the EML4-ALK fusion protein, respectively, has heralded the era of truly personalized medicine for patients with solid tumors. Nonetheless, there is recognition that genetic alteration alone does not always result in a given disease phenotype, and the role of epigenetic regulation of gene transcription is also seen as increasingly critical. Indeed, normal and/or aberrant cellular behavior is likely a consequence of an integrated influence of both genomic and epigenomic aspects.

Epigenetic modulation, classically defined as "the heritable transmission of phenotype without a change in the underlying DNA sequence", can be considered as a series of complex, interrelated, and dynamic set of stable post-translational alterations that control access of transcriptional machinery to DNA and thereby determine cell function. The term "histone code" has been invoked to describe the set of modifications or "marks" that specify the sequences of DNA that are actively transcribed or silenced. Chemically, these post-translational alterations involve either cytosine methylation and hydroxymethylation on DNA or diverse reversible histone tail modifications including acetylation, methylation, phosphorylation, ubiquitinilation, etc., which result in physical effects such as nucleosome positioning (Figure 1). A common model is that transcription factors in complex with histone reader proteins recruit the enzymes that catalyze these modifications, which, in concert, regulate gene expression. Several recent reviews describe in detail the roles and mechanisms that these modifications play in various disorders, although it is important to recognize that much of the underlying data are still emergent, and new interpretations challenging current hypotheses are common.

Until recently, epigenetic drug discovery efforts were confined to inhibiting DNA methyltransferases (DNMT) and histone deacetylase enzymes (HDAC). The nucleoside analogue azacytidine and its deoxy derivative decitabine are both irreversible DNMT inhibitors and approved for treating myelodysplastic syndrome. In the HDAC inhibitor area, the hydroxamic acid vorinostat and the macrocyclic disulfide romidepsin are indicated for cutaneous T-cell lymphoma. Although effective in such hematologic malignancies, these medicines generally suffer from a suboptimal therapeutic index largely due to poor selectivity for the molecular target subtypes but also often combined with more pleiotropic off-target activities. This highlights a fundamental issue for many of the currently known epigenetically targeted drugs, and substantial medicinal chemistry efforts continue to optimize, for example, HDAC isoform selectivity as an approach to maximizing desirable pharmacology while minimizing toxicity. Ultimately, it remains to be determined whether absolute selectivity is achievable or even compatible with a therapeutic effect given the level of redundancy in function of many HDACs.

The four currently approved epigenetic drugs were discovered using phenotypic assays and without a priori knowledge of their molecular target, but the next generation of epigenetic agents now in clinical development target chromatin in a more specific manner. Remarkably, rather than directly promoting or inhibiting histone tail functionalization, compounds such as I-BET762 and JQ1 block the protein:protein interaction between acetylated lysine residues on the histone tail and subtypes of bromodomain "reader" proteins. High-resolution crystal structures of many bromodomains proteins are now routinely available. Increasingly, these are often in complex with small molecule ligands, not only allowing rationalization of the binding modes and selectivity profiles for diverse chemical templates but also enabling the medicinal chemistry-led design of novel compounds to enhance selectivity and druglike molecular properties (Figure 2). Biologically, because bromodomains can serve to recruit and localize components of transcriptional apparatus to specific gene loci, their inhibition can result in effective silencing of the expression of these genes. Moreover, despite intuitive expectations that changes in expression of thousands of genes would be induced, early experience with bromodomain antagonists in macrophages and cancer cells indicates the potential to control expression of somewhat smaller subsets of disease-related genes. It is very likely that as additional compounds are discovered, the rich biology associated with blockade of acetyl reader proteins will be better understood and exploited for therapy. It is also pertinent to note that bromodomains are part of a larger family of proteins that bind to various histone marks, and undoubtedly, some of these will also be amenable to small molecule modulation. In fact, a recent report of a simple acylpyridine derivative able to block binding of the methyllysine mark reader L3MBTL1 strongly suggests the scope for the design of additional, higher affinity analogues for this large subfamily of reader proteins. Compelling disease association and/or target validation will be key checkpoints in the development of nonbromodomain chromatin mark reader protein antagonists, and the availability of tool compounds to decipher the physiological role of these proteins will certainly be critical.

Progress in the advancement of modulators of "writer" proteins to the clinic has been slower. There is intense interest in finding selective, catalytic inhibitors of members of the histone methyltransferase family, several of which are thought to be vital players in regulating fundamental cellular differentiation programs as well as the initiation and progression of

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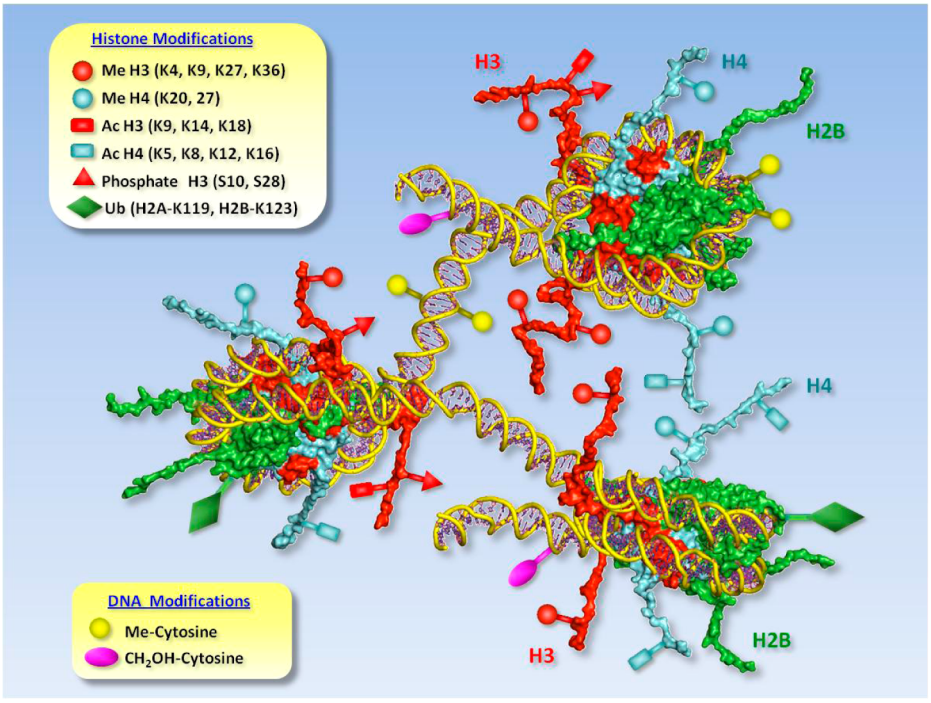

Figure 1. Structure of nucleosome particles showing the DNA helix (yellow backbone) wrapped 1.7 turns around a core consisting of eight histone molecules, two each of H2A, H2B (green), H3 (red), and H4 (cyan). Histone tails are represented in their extended conformations, to illustrate the wide range of epigenetic modifications possible (i.e., methylation, acetylation, phosphorylation, and ubiquitination). The figure was generated using PDB files 1KX5 and 1ZBB.

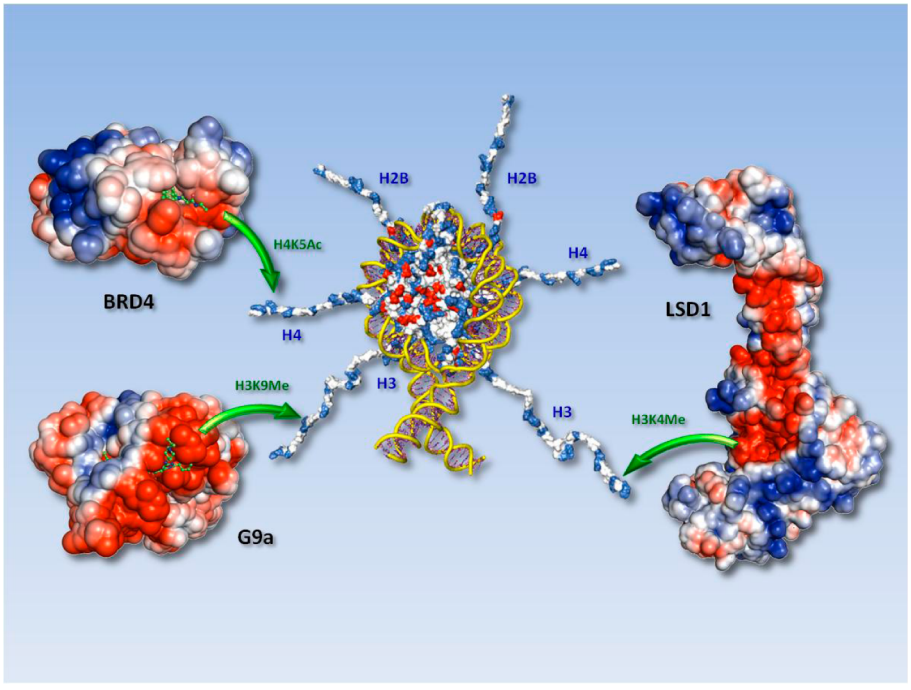

Figure 2. Example of three different epigenetic targets recently crystallized in complex with small molecule inhibitors, including a reader (BRD4/I-BET762 complex, 3P5O), a writer (G9a/UNC-0638/SAH ternary complex, 3RJW), and an eraser (LSD1/FAD-tranylcypromine adduct, 2UXX). The three proteins are shown as their electrostatic potential surfaces, to illustrate the intensely negatively charged region of their binding pockets, complementing the positively charged nature of the histone tails. Electrostatic potential surfaces were calculated by APBS, with ranges from +2.5 (blue) to −2.5 (red) kT/e; shown is the value as mapped onto the Connelly solvent accessible surfaces, using PYMOL (1.4 Å probe radius).

cancer. The methyltransferases sequentially transfer methyl groups from the cofactor *S*-adenosylmethionine (SAM) to the terminal amine of specific substrate lysine and/or arginine residues. The transformation, generating *S*-adenosylhomocysteine and a methylated histone, has a clear analogy to a more familiar protein kinase-catalyzed reaction generating adenosine diphosphate and a phosphoprotein. Given this, there has been a rush to exploit the extensive learnings from designing ATP-

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competitive kinase inhibitors to the corresponding conserved SAM-binding pocket of the methyltransferases. Crucially, the availability of crystal structures of histone methyltransferases with bound cofactor and/or peptide substrates has allowed for detailed analysis of binding modes and enabled rational design of selective inhibitors. Equally, the more traditional approach using random screening of large compound collections has also met with some, albeit more limited, success. Regardless of their origin, the ability to obtain highly selective, peptide or SAM-competitive inhibitors of several histone methyltransferases (e.g., DOT1L and EZH2) certainly establishes druggability of the enzymes. Early data from the use of some of these compounds in cells and in animal studies are very encouraging and consistent with a histone code hypothesis in which downstream gene transcription is selectively altered without overt, acute toxicity.

As mentioned above in connection with HDACs, proteins designed to reverse the action of writer enzymes have evolved to help maintain overall epigenetic fidelity as well as to retain the dynamic nature of the histone code. Many epigenetic marks may be relatively transient and are presumably removed by specific and/or general hydrolase enzymes. However, the N-methylation of lysine or arginine residues of the histone tail introduces a more stable mark requiring the action of dealkylating enzymes to revert to the basal, primary amine state. The two families of proteins known to carry out this function either rely on an iron(II)/α-ketoglutarate redox system or use flavin adenine dinucleotide as a cofactor. Knowledge of the molecular mechanisms of these transformations has allowed medicinal chemists to design potent, reversible as well as irreversible, suicide inhibitors. The first of these are close to entering clinical trials for the treatment of Huntington's disease, a debilitating neurodegenerative disorder affecting muscle function. The discovery of potent, selective inhibitors of the Jumonji family of α-ketoglutarate histone demethylases has progressed slowly, although recently reported JMJD2 inhibitors designed by employing biophysical and structural approaches is noteworthy. Furthermore, consistent with the view of a close cross-talk between genetic and epigenetic effects driving disease is the recent observation in acute myeloid leukemia patients of activating somatic mutations in the metabolic enzyme isocitrate dehydrogenase leading to downstream inactivation of epigenetic enzymes such as the TET family of 5-methylcytosine hydroxylase. In this context, it is reasonable to speculate that inhibitors of mutant isocitrate dehydrogenase and of DNMTs (or other histone demethylases) might be synergistic.

Spurred by the dramatic discovery of bromodomain antagonists, the landscape for the development of second generation chromatin modifying drugs is evolving very rapidly. The challenge of identifying and optimizing hits and leads for an array of histone tail interacting proteins is evidently surmountable. Selectivity of compounds within closely related families remains to be demonstrated for all of the targets investigated, although preliminary data indicate a surprising lack of general cross-reactivity. Notwithstanding these positive developments, the current focus of novel agents remains in cancer indications. Concern about developing molecules with sufficient target specificity and, in turn, transcriptional selectivity is receding based on an increased understanding of and experience with both established drugs and novel agents. In oncology, recognition of the important cross-talk between the genome and the epigenome as a driver of many cancers is gaining acceptance, and targeting of chromatin modifiers is seen as a credible approach to novel therapeutics. In this context, the potential of altering the functional activity of potent oncogenes such as Myc is particularly exciting, and attention is rapidly moving toward gaining a better understanding of how best to use these agents in the clinic. Outside of cancer, regulation of the secondary, chronic phase of the immune response with bromodomain inhibitors is most promising and clinical investigation is warranted. In addition, roles for epigenetic changes in psychiatry disorders, neurodegenerative diseases and even in re-emergence of latent viral infections continue to surface. Nonetheless, major issues remain to be addressed before these newer epigenetic agents become established therapeutics particularly in chronic, nonlife-threatening diseases. These include determination of long-term and potentially heritable epigenetic changes manifesting as unanticipated toxicities even after ceasing therapy.

In summary, the future for manipulating gene transcription via the histone code in a controlled and directed manner by modulation of chromatin binding proteins with small molecules is bright. The area is poised for some extraordinary developments in understanding both the basic science of epigenetics and the clinical utility of novel therapeutics.

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Notes

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