DOI: 10.1002/anie.201101414

Chromatin Disruptors

Disrupting the Reader of Histone Language

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bromodomains · epigenetics · histone code · inhibitors · small molecules

Research in chromatin and epigenetics is rapidly expanding, and with this growth opportunity come for the development of novel therapeutics that target newly identified players. Although the human genome is a blueprint of cellular potential, an additional layer of information, the epigenome, ultimately controls whether particular genes are expressed in their appropriate context.[1] The epigenome consists of a complex array of specific chemical marks on chromatin (the chromosomal DNA–protein complex), which can dynamically regulate gene expression.[1b] These chromatin-based mechanisms are being revealed and recent evidence suggests that targeting these pathways holds great promise for therapeutic interventions of human disease and afflictions.

Chromatin consists of histone proteins that spool the chromosomal DNA and can—depending on the packing—restrict access to protein machines that transcribe the DNA sequence into mRNA.[2] Posttranslational modifications (PTMs; e.g. phosphorylation, acetylation, and methylation) on histones regulate the accessibility of DNA through a number of mechanisms. The combination of PTMs in the proper context is thought to give rise to a histone code or histone language that is interpreted by an array of diverse proteins.[3] These proteins can be divided into three broad classes of "writers", "erasers", and "readers". The writers and erasers of histone PTMs are enzymes such as acetyltransferases, deacetylases, kinases, phosphatases, methyltransferases, and demethylases. Among the protein readers of this histone language is the bromodomain family that recognizes acetylated lysine residues in histones.[4] There are 57 bromodomains found among 41 different human proteins. The bromodomain-and-extraterminal (BET) subfamily is represented by BRD2, BRD3, BRD4, and BRDT.[5] This subfamily shares similar domain architecture with two highly conserved amino-terminal bromodomains. Two recent studies report the development of small-molecule compounds that specifically disrupt the interactions between these bromodomains and acetylated histone tails.[6] These results provide compelling proof-of-concept that disrupting histone readers is a viable strategy for the development of epigenetic drugs.

Filippakopoulos et al. describe a cell-permeable small molecule, (+)-JQ1, that binds bromodomains and competes with acetyl-lysine-modified histone peptides.[6a] (+)-JQ1 is a novel thienotriazolo-1,4-diazepine that contains a *tert*-butyl ester at C6 and binds a subset of human bromodomains with nanomolar affinity (Figure 1a). Similar to interactions found in acetyl-lysine complexes of bromodomains, the triazole ring of (+)-JQ1 formed a hydrogen bond with an evolutionarily conserved asparagine (Asn140 in BRD4 and Asn429 in BRD2). Conserved BET residues in the ZA- and BC-loop regions also contribute to ligand binding in both BRD domains (Figure 1b). Owing to steric clashes with residues of the ZA-loop region of BRD2 the (−)-JQ1 enantiomer resulted in an energetically unfavorable binding conformation.

The gene that codes for BRD4 is affected by a recurrent t(15;19) chromosomal translocation found in aggressive human squamous carcinoma.[7] This translocation results in a fusion gene that expresses a fusion protein of the tandem N-terminal bromodomains of BRD4 and the nuclear protein in testis (NUT). The BRD4-NUT oncoprotein defines the NUT midline carcinoma (NMC), which is a fatal malignancy. This genetically defined condition offers a unique opportunity to investigate whether small-molecule disruptors can afford the desired effect of blocking the action of an oncogenic chromatin reader. A human cell line was treated with (+)-JQ1 and fluorescence recovery after photobleaching (FRAP) experiments were performed to assess the mobility of the GFP-tagged version of BRD4 (GFP = green fluorescent protein). The authors concluded that (+)-JQ1 displaces chromatin-bound GFP-BRD4 because the observed recovery is immediate in the presence of (+)-JQ1. In other experiments, the authors treated a patient-derived NMC cell line with (+)-JQ1 (500 nM) and observed the loss of characteristic nuclear foci containing the NUT protein. In several BRD4-dependent NMC cells, treatment with (+)-JQ1 induced differentiation and growth arrest. These observations make a compelling case that (+)-JQ1 disrupts the reader BRD4 and prevents oncogenetic progression. To provide in vivo support, the authors examined several patient-derived xenograft models. Mice with established tumors were treated with (+)-JQ1 by means of daily intraperitoneal injection. The authors found (+)-JQ1 to be well tolerated with marked tumor regression and prolonged survival after 18 days of therapy.

Utilizing a high-throughput screen for activators of a ApoA1-luciferase reporter in HepG2 cells, Nicodeme et al. identified I-BET, an optimized derivative of a benzodiazepine compound (Figure 1a).[6b] The authors demonstrated that I-

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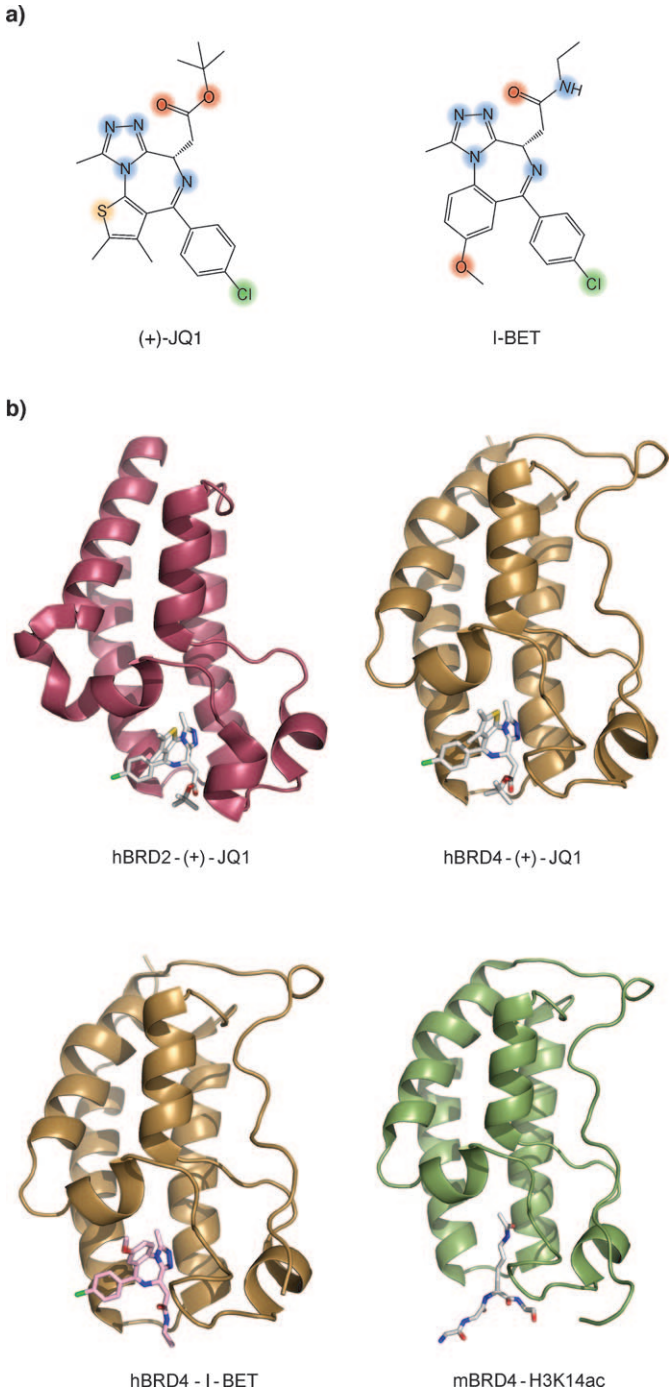

Figure 1. Small-molecule disruptors/inhibitors of epigenetic regulators. a) (+)-JQ1 and I-BET target the BET family of bromodomains. Important features of the compounds are highlighted. b) The crystal structures of human BRD2 and BRD4 in complex with JQ1 and I-BET. mBRD4 in complex with H3K14ac is included for comparison (bottom right).[13] Crystallographic information was obtained using the following PDB ID accession numbers (in order from left to right and from top to bottom): 3ONI, 3MXF, 3P5O, 3JVK.

BET targets inflammatory gene expression by disrupting the interaction of acetylated histones with the BET family of chromatin readers. The compound binds BRD2, BRD3, and BRD4 similarly with low nanomolar affinity, and much like in the case of JQ1, its enantiomer does not bind members of the BET subfamily. The triazole ring of I-BET imitates essential interactions between acetyl-lysine, Asn140, and Tyr97 (Figure 1 b). The ZA hydrophobic channel and WPF shelf outside of the acetyl-lsyine binding pocket further determine I-BET selectivity by imposing spatial constraints on the size of molecules that can interact.

Using a mouse model of immune-responsiveness, Nicodeme et al. found that I-BET treatment provided protection against lipopolysaccharide-induced endotoxic shock and bacteria-induced sepsis.[6b] The impact of I-BET on LPS-inducible gene expression was highly selective. Gene expression analyses and epigenetic profiles at a number of LPS-inducible genes revealed that I-BET-sensitive gene repression is characterized by low basal levels of histone H3 and H4 acetylation, trimethylation of Lys4 on histone H3 (H3K4me3), RNA polymerase II, and low CpG content at their promoters. Higher basal levels of histone H3 and H4 acetylation at unaffected I-BET gene promoters suggest that these genes are already primed or actively transcribing. Although a number of mechanistic questions still remain, these results suggest the possibility of a new generation of drugs that modulate inflammatory response by disrupting readers of the histone language.

These two new small molecules, (+)-JQ1 and I-BET, are encouraging candidates for new therapeutic approaches that directly target readers of chromatin modifications. They offer compelling proof-of-concept that such drugs are possible. Targeting the epigenome as a viable drug strategy is supported by the current FDA drugs (Figure 2) Vorinostat

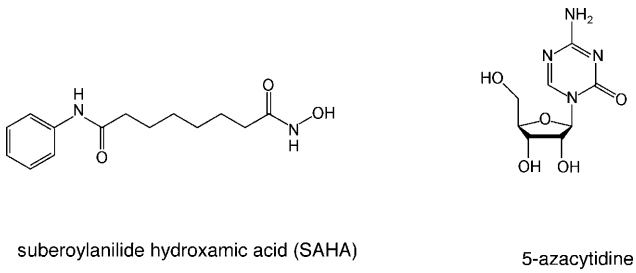

Figure 2. Structures of SAHA and Azacitidine (5-azacytidine).

(or suberolyanilide hydroxamic acid (SAHA)) and Azacitidine (or 5-azacytidine).[8] SAHA is an inhibitor of histone deacetylases (HDACs) and is used to treat cutaneous T-cell lymphoma.[9] Azacitidine is incorporated into DNA and prevents methylation; it is used as a chemotherapeutic towards myelodysplastic syndromes.[10] Both of these drugs essentially act as enzymes inhibitors, a well-established approach in drug development. Though the exact mechanism of action is unknown, it is believed to involve the transcriptional derepression of tumor-suppressor genes.[11]

In the case of SAHA, it is still unclear which deacetylases are appropriately inhibited in cells and which deacetylase substrates are involved. HDACs regulate the acetylation status of many proteins.[12] It may be that the combined effect on several HDACs and multiple substrates is a key to efficacy. This possibility makes drug improvement through directed

structure–activity relationships problematic. Similar concerns may apply in the case of I-BET, whose specific mechanism of action is still vague. In the case of JQ1-based compounds to treat NMC, the BRD4-NUT oncoprotein is defined genetically, allowing a clearer path to investigate the structure–activity relationships between BRD4-NUT and improved compounds. The rather remarkable structural similarities of JQ1 and I-BET (Figure 1a) suggest that these compounds could function interchangeably. To investigate target overlap, it would be interesting if these compounds were examined as both inflammation and NMC suppressors.

It now seems inevitable that intensive efforts will be invested toward the identification of small molecules that specifically disrupt chromatin-binding proteins gone awry, such as those defined by expressed translocations or inappropriate overexpression. Such investigations could lead to new therapeutics; at the very least the research community can utilize these tools to investigate how chromatin dynamics regulate gene expression. The recent success of BET disruptors may signal a potential paradigm shift towards inclusion of readers in the pool of writers and erasers in the design and development of epigenetic modulators.

Received: February 25, 2011
Published online: May 25, 2011

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