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Disrupting the Reader

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Research in chromatin and epigenetics is rapidly expanding, and with this growth comes opportunities 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^[1a,b]. The epigenome consists of a complex array of specific chemical ‘marks’ on chromatin (the 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 (core histones H2A, H2B, H3 and linker histones) that spool DNA and can restrict access to protein machines that transcribe the DNA sequence into functional gene products^[2a,b]. Post-translational 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 read and interpreted by an array of diverse proteins^[3a,b]. These proteins can be divided into three broad classes of ‘writers’, ‘erasers’, or ‘readers’. The so-called ‘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 are the bromodomain family that function as acetyl-lysine recognition motifs^[4a,b]. There are 57 bromodomains found among 41 different human proteins. The bromodomain and extra-terminal (BET) subfamily are represented by BRD2, BRD3, BRD4 and BRDT^[5]. The BET family of bromodomains share a 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^[6a,b]. 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 thieno-triazolo-1,4-diazepine that contains a *t*-butyl ester at C6 and binds a subset of human bromodomains with nanomolar affinity (Figure 1a). Similar to interactions found in acetyl-lysine complexes, the triazole ring of (+)-JQ1 formed a hydrogen bond with an evolutionarily conserved asparagine (Asn 140 in BRD4 and Asn 429 in BRD2). Conserved BET residues in the ZA- and BC-loop regions also contributed to ligand stabilization in both

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BRD domains (Figure 1b). Due to steric clashes with residues of the ZA-loop region the (–)-JQ1 enantiomer resulted in an energetically unfavorable binding conformation.

BRD4 is part of a recurrent t(15;19) chromosomal translocation found in aggressive human squamous carcinoma [7a–c]. These translocations express 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 offered a unique opportunity to investigate whether small molecule disruptors could 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 fluorescently tagged version of BRD4 (GFP–BRD4). The authors concluded that (+)-JQ1 displaced chromatin bound GFP–BRD4 because the observed recovery is immediate in the presence of (+)-JQ1. In other experiments, the authors treat 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 made a compelling case that (+)-JQ1 was disrupting the reader BRD4 and preventing oncogenetic progression. To provide in vivo support, the authors examined several patient-derived xenograft models. Mice with established tumors were treated with (+)-JQ1 via 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 describe the identification of I-BET, an optimized derivative of a benzodiazepine compound [6b]. The authors demonstrated that I-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 (–)-JQ1, the enantiomeric form had no activity towards BET. The triazolyl ring of I-BET imitated essential interactions between the acetyl-lysine, asparagine-140 and tyrosine-97 (Figure 1b). The ZA hydrophobic channel and WPF shelf outside of the acetyl-lysine 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, 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 I-BET-sensitive gene repression was characterized by low basal levels of histone H3 and H4 acetylation, trimethylation of Lys4 on histone H3 (H3K4me3), RNA Pol II, and low CpG content at their promoters. Higher basal levels of histone H3 and H4 acetylation at unaffected I-BET gene promoters suggested that these genes were 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.

Two new small molecules, (+)-JQ1 and I-BET, are encouraging prospects 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 current FDA drugs, Vorinostat or suberoylanilide hydroxamic acid (SAHA), and Azacitidine [8] (Figure 1a). SAHA is an inhibitor of histone deacetylases (HDACs) and is used to treat cutaneous T cell lymphoma [9a,b]. 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's effectiveness, 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–B) suggest that these compounds could function interchangeably. To investigate target overlap, it would be interesting if these compounds were examined as both inflammatory and NMC suppressors.

It now seems inevitable that intense 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 over-expression. 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' when designing and developing epigenetic modulators.

References

1. a) Egger G, Liang G, Aparicio A, Jones PA. *Nature*. 2004; 429:457–463. [PubMed: 15164071] b) Goldberg AD, Allis CD, Bernstein E. *Cell*. 2007; 128:635–638. [PubMed: 17320500]
2. a) Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. *Nature*. 1997; 389:251–260. [PubMed: 9305837] b) Luger K. *Curr Opin Genet Dev*. 2003; 13:127–135. [PubMed: 12672489]
3. a) Gardner KE, Allis CD, Strahl BD. *J Mol Biol*. 2011b) Oliver SS, Denu JM. *Chembiochem*. 2011; 12:299–307. [PubMed: 21243717]
4. a) Mujtaba S, Zeng L, Zhou MM. *Oncogene*. 2007; 26:5521–5527. [PubMed: 17694091] b) Sanchez R, Zhou MM. *Curr Opin Drug Discov Devel*. 2009; 12:659–665.
5. Zeng L, Zhou MM. *FEBS Lett*. 2002; 513:124–128. [PubMed: 11911891]
6. a) Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, Cameron MJ, Schwartz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S, Bradner JE. *Nature*. 2010; 468:1067–1073. [PubMed: 20871596] b) Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, Chung CW, Chandwani R, Marazzi I, Wilson P, Coste H, White J, Kirilovsky J, Rice CM, Lora JM, Prinjha RK, Lee K, Tarakhovsky A. *Nature*. 2010; 468:1119–1123. [PubMed: 21068722]
7. a) French CA, Miyoshi I, Aster JC, Kubonishi I, Kroll TG, Dal Cin P, Vargas SO, Perez-Atayde AR, Fletcher JA. *Am J Pathol*. 2001; 159:1987–1992. [PubMed: 11733348] b) Engleson J, Soller M, Panagopoulos I, Dahlen A, Dictor M, Jerkeman M. *BMC Cancer*. 2006; 6:69. [PubMed: 16542442] c) French CA, Miyoshi I, Kubonishi I, Grier HE, Perez-Atayde AR, Fletcher JA. *Cancer Res*. 2003; 63:304–307. [PubMed: 12543779]
8. Marks PA, Xu WS. *J Cell Biochem*. 2009; 107:600–608. [PubMed: 19459166]
9. a) Duvic M, Vu J. *Expert Opin Investig Drugs*. 2007; 16:1111–1120. b) Marks PA, Breslow R. *Nat Biotechnol*. 2007; 25:84–90. [PubMed: 17211407]
10. Keating GM. *Drugs*. 2009; 69:2501–2518. [PubMed: 19911860]
11. Xu WS, Parmigiani RB, Marks PA. *Oncogene*. 2007; 26:5541–5552. [PubMed: 17694093]

12. Haberland M, Montgomery RL, Olson EN. Nat Rev Genet. 2009; 10:32–42. [PubMed: 19065135]
13. Vollmuth F, Blankenfeldt W, Geyer M. J Biol Chem. 2009; 284:36547–36556. [PubMed: 19828451]

Figure 1.

Small molecule disruptors/inhibitors of epigenetic regulators. A) The structures of (+)-JQ1, I-BET, SAHA and 5-azacytidine. JQ1 and I-BET target the BET family of bromodomains. Important atomic features of JQ1 and I-BET are highlighted for comparison; oxygen=red, nitrogen=blue, chlorine=green, sulfur=yellow B) The crystal structures of BRD2 and BRD4 in complex with JQ1 and I-BET. mBRD4 in complex with H3K14ac is included for comparison (far right)^[13]. Crystallographic information was obtained using the following PDB ID accession numbers (in order from left to right): 3ONI, 3MXF, 3P5O, and 3JVK.