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Targeting Chromatin Readers

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Abstract

Modulation of gene expression through epigenetic signaling has recently emerged as a novel approach in treating human disease. Specifically, chromatin reader proteins, which mediate protein–protein interactions via binding to modified lysine residues, are gaining traction as potential therapeutic targets. Herein, we review recent efforts to understand and modulate the activity of chromatin reader proteins with small-molecule ligands.

Therapies resulting from exploitation of signal transduction targets— largely from the protein kinase target class—are a crowning achievement from the “pregenomic” era of cancer biology and drug discovery. This successful paradigm was based on a gradual progression over 20 years from understanding the role of kinases as oncogenes to their establishment as druggable targets with clinically useful levels of efficacy and selectivity. This foundation has rendered any new potential kinase target in cancer (or other diseases) readily assailable for drug discovery with proven technologies. In the “post-genomic” era, tumor genome sequencing efforts such as the Cancer Genome Atlas are cataloging additional genetic events that cause or sustain human cancers and have identified tractable, novel targets for therapy (e.g., B-RAF-vemurafinib and Alk-crizotinib). Of note, such efforts have clearly shown epigenetic phenomena to be critical for tumor maintenance: a summary of Cancer Genome Atlas efforts to date (B. Vogelstein, NCI Translational Science Meeting, 28 July 2011) revealed that 10 of 12 newly identified oncogenes were directly related to regulation of chromatin function. Chromatin modifiers represent a relatively underexplored area for drug discovery; few potent and selective small-molecule ligands for these targets exist, but the potential of this area to impact therapeutics may rival that of the protein kinase target family. This raises the question of whether we can build a viable approach to establish the clinical utility of these targets in less than the 20 years required for protein kinases.

Chromatin is the complex of histone proteins, DNA, and RNA that efficiently packages the genome in an appropriately accessible state within each cell. The state of chromatin, and therefore access to the genetic code, is largely regulated by specific chemical modifications to histone proteins and DNA, as well as the recognition of these marks by other proteins and

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CONFLICT OF INTEREST

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protein complexes (Figure 1). The chemical modification of chromatin is carried out by families of enzymes that can both “write” (create a posttranslational modification (PTM)) and “erase” (chemically remove) such PTMs. These enzymes include druggable targets such as protein kinases and histone deacetylases, and there is also much recent excitement in the area of inhibitor discovery for protein lysine methyltransferases. Although enzymes are frequently favored as targets for drug discovery because of the precedent for medicinal chemistry success and the ligand design information inherent in the chemical transformations they perform, these chromatin-modifying enzymes also frequently create a binding site for the recruitment of other proteins. Targeting the readers of chromatin PTMs represents a novel emerging area of drug discovery focus that may prove useful in modulating both chromatin state and the activity of epigenetic writers and erasers, which also frequently depend on existing PTMs to recognize their substrates, with a unique pharmacology as compared with enzyme inhibitors.[1] We present a summary of recent efforts aimed at modulating the activity of chromatin reader proteins of modified lysine via small-molecule intervention with the goal of highlighting this less precedented landscape of viable epigenetic targets.

ACETYL-LYSINE “READER” INHIBITION

Acetylation of lysine by histone acetyl transferases eliminates the residue’s positive charge and creates a binding motif for the recruitment of bromodomain-containing regulators of transcription. There are 61 structurally homologous bromodomains in the human genome, and recently potent and selective small-molecule ligands have been reported for the bromodomain-containing (BRD) subfamily of these domains, also known as bromo and extra-terminal (BET) proteins. The first well-characterized inhibitors of this class, JQ1 and I-BET, which contain thienodiazepine and benzodiazepine core structures, respectively, were reported contemporaneously and shown to bind BET-bromodomains with sub–100 nM affinity, with a clear mode of action as elucidated by crystallographic studies (Figure 2a). These discoveries were crucial in validating protein–protein interactions triggered by PTMs as tractable, and generated excitement that other families of “reader” proteins may be amenable to selective small-molecule intervention as well.[1]

Nuclear protein in testes (NUT) midline carcinoma is a rare and aggressive form of cancer in which a translocation results in the fusion of BET-bromodomains to an unrelated protein, NUT. This fusion protein leads to hyperacetylation of inactive chromatin domains and inactivation of p53. Inhibition of BRD4-NUT chromatin binding by JQ1 results in cell differentiation, cell-cycle arrest, and induction of apoptosis in NUT midline carcinoma cell lines. Furthermore, BRD4-NUT mouse xenograft tumor models demonstrated impressive tumor shrinkage and increased survival on treatment with JQ1.

BRD4 was also recently associated with acute myeloid leukemia via an RNA interference screen and shown to be critically required for the maintenance of this aggressive hematopoietic malignancy.[2] Indeed, JQ1 led to pronounced antileukemic effects in a variety of human acute myeloid leukemia cell lines and patient samples. The inhibitor was then used to identify bromodomain proteins as regulatory factors for c-Myc, the prototypical undruggable oncoprotein, presenting BRD4 antagonism as an appealing strategy for the

downregulation of c-Myc in certain cancers. In various models of multiple myeloma, a Myc-dependent hematological malignancy, inhibition of BRD4 by JQ1 was effective at downregulating the Myc-dependent transcriptional network and resulted in significant antiproliferative effects, cell-cycle arrest, and cellular senescence. These findings help to validate epigenetic "reader" machinery as targets in cancer therapy and support the utility of drug-like bromodomain inhibitors as therapeutic agents, particularly in Myc-dependent hematopoietic cancers.

METHYL-LYSINE "READER" INHIBITION

There are more than 200 methyl-lysine (Kme) reader domains described within several protein families: plant homeo domains; the so-called "royal family" made up of Tudor, Agenet, chromo, PWWP, and malignant brain tumor (MBT) domains; and the WD40 repeat proteins consisting of WDR5 and EED. Unlike acetylation, lysine methylation is a subtle modification that preserves the positive charge on lysine, and, remarkably, the addition of a single methyl group can have profound effects on gene expression. A unifying feature of these domains is the existence of an aromatic cage that comprises the Kme binding pocket, facilitating recognition of the methyl-ammonium group via cation-π, hydrogen bond, and van der Waals interactions.[2] As the recognition of methyl marks is a cornerstone of chromatin regulation, Kme readers are also of interest as potential therapeutic targets. For example, genomic translocations resulting in hybrid Kme reader domains with altered chromatin binding properties have been associated with many cancers.[3] Wagner and co-workers[4] recently reported on the development of a screening strategy for small-molecule inhibitors of lysinespecific demethylase 5A (JARID1A), one such Kme reader that participates in these aberrant genetic fusion proteins. Small-molecule inhibitors of JARID1A were identified albeit with modest affinity relative to its native peptide substrate, H3K4me3.

Our laboratory has also made progress toward potent small-molecule inhibitors of the family of MBT domain–containing proteins. The MBT domain family was appealing as an initial focus due to its tractable size (nine human proteins contain MBT domains), abundant structural information, and prospective druggability. MBT domains contain a narrow pocket that envelops the modified lysine side-chain facilitating binding to mono-and/or dimethyl-lysine (Kme1 and Kme2) via a "cavity insertion" mode of Kme recognition. MBT domain–containing proteins have also been functionally associated with important regulatory changes in the transcriptional state of chromatin regions and significant developmental biology. For example, the three MBT repeat fragment of human lethal 3 MBT-like protein-1 has been reported to compact nucleosomal arrays within the context of binding H4K20me1-2, leading to gene repression.

Using structure-based design methods, we recently discovered UNC669, a small molecule that antagonizes the Kme binding function of lethal 3 MBT-like protein-1, and generated the first small-molecule, methyl-lysine binding domain cocrystal structure (Figure 2b).[4] UNC669 contains a piperidine–pyrrolidine moiety that serves to anchor the compound into the Kme binding site by appropriately filling the aromatic cage and maximizing van der Waals contacts while maintaining the cation-π and hydrogen bond interactions required for recognition.

The unique recognition mode of Kme reader proteins and the lack of known high-affinity ligands has prompted us to undertake a target class approach toward the parallel discovery of molecular probes for members of this family. In this approach, all synthetic Kme mimics are screened against a panel of proteins from the MBT domain family and other Kme readers. By increasing the breadth of our Kme reader assay panel, the chances of finding potency- and selectivity-enhancing features in synthetic ligands is increased via testing each ligand hypothesis vs. a large number of functionally homologous but structurally distinct binding sites. Thus far, this method of ligand discovery has proven to be fruitful and has led to the recent discovery of UNC1215, a first-in-class chemical probe for the Kme1,2 reading function of lethal 3 MBT-like protein-3 (ref. 5). Our panel is also biased to include Kme reader proteins that have been linked to specific disease states, increasing the chances that a potent chemical probe could eventually lead to a compound of true therapeutic value.

SUMMARY

Because the biological consequences of inhibiting chromatin reader domains are still unknown in most cases, target validation of epigenetic regulators with chemical probes such as JQ1 is critical to allow the pharmaceutical industry to focus its efforts on the most promising points for therapeutic intervention. Furthermore, it is increasingly clear that the modulation of certain nonenzymatic epigenetic mechanisms has the potential to overcome undruggable genetic changes that drive cancer, such as the overexpression of c-myc.[1] The pursuit of high-quality chemical probes for chromatin PTM enzymes and reader proteins represents a novel and emerging area that we believe will open new avenues of research in chromatin biology and, in time, translate to therapeutic value comparable with inhibitors of protein kinases.

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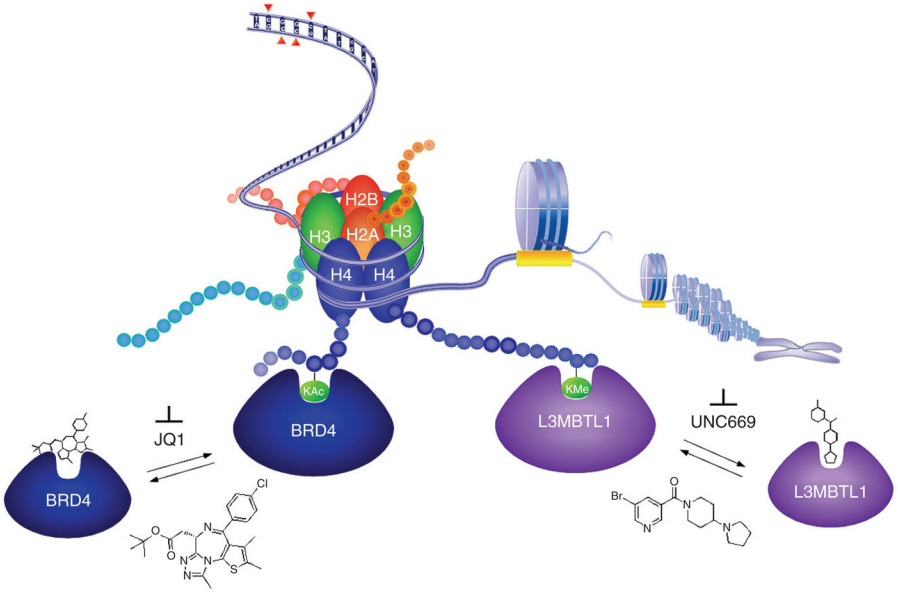

Figure 1.

The basic functional unit of chromatin is the nucleosome, a histone octamer around which DNA is wrapped. Lysine residues on the histone tail are subject to posttranslational modifications including methylation and acetylation (green). Reader proteins that recognize methyl- and acetyl-lysine on the amino-terminal tail of histone 4 include lethal 3 MBT-like protein-1 (L3MBTL1; purple) and bromodomain-containing-4 (BRD4; blue), respectively. Small molecules such as UNC669 and JQ1 target the peptide binding pockets of these reader proteins and displace them from chromatin.

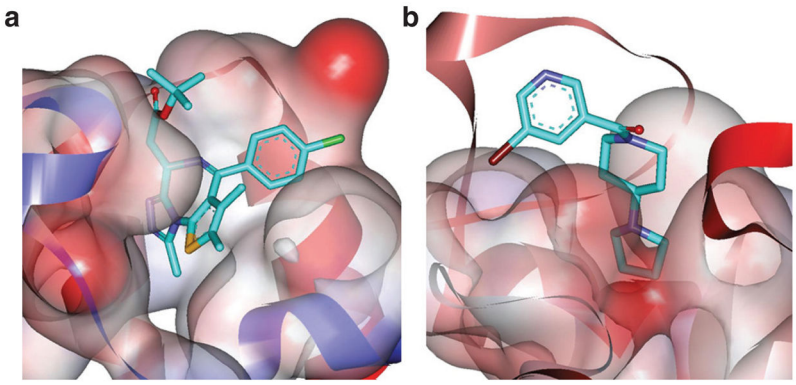

Figure 2.

Cocrystal structures of methyl-lysine and acetyl-lysine reader inhibitors. (**a**) JQ1 is shown in complex with the acetyl-lysine reader, bromodomain-containing-2 (BRD2) (Protein Data Bank identification code: 3ONI). (**b**) UNC669 is shown in complex with the methyl-lysine reader, lethal 3 MBT-like protein-1 (L3MBTL1) (Protein Data Bank identification code: 3P8H).