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Using Chemical Epigenetics to Target Cancer

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SUMMARY

Transcription is epigenetically regulated by the orchestrated function of chromatin binding proteins that tightly control the expression of master transcription factors, effectors and supportive housekeeping genes required for establishing and propagating the normal and malignant cell state. Rapid advances in chemical biology and functional genomics have facilitated exploration of targeting epigenetic proteins, yielding effective strategies to target transcription while reducing toxicities to untransformed cells. Here, we review recent developments in conventional active site and allosteric inhibitors, peptidomimetics, and novel PROTAC technology that have deepened our understanding of transcriptional processes and led to promising preclinical compounds for therapeutic translation, particularly in cancer.

eTOC blurb

New methods for targeting epigenetic regulatory proteins have led to rapid improvements in the mechanistic understanding of cancer biology and provided novel approaches for cancer therapies.

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AUTHOR CONTRIBUTIONS

A.D.D and J.Q. determined the content. All authors were involved in the writing and editing of the manuscript.

SUPPLEMENTAL INFORMATION

All chemical structures in this review and their associated references are listed in Supplemental Table 1. Supplementary discussion is provided in a supplementary note.

DECLARATIONS OF INTERESTS

J. Q. is scientific co-founder and consultant for Epiphanes, and share holder of Zentalis.

This review summarizes these new developments with a focus on regulation of transcription, and discusses fundamental challenges and future directions for cancer chemical epigenetics.

Keywords

Transcription; Epigenetics; Inhibitor; Degrader; Chromatin; PROTAC; peptidomimetic

INTRODUCTION

Many types of cancer cells display selective addiction to transcriptional processes that play critical roles in driving their malignant behavior. This process is tightly controlled by networks of epigenetic proteins and is required for the maintenance of normal cell growth, induction of differentiation, and in disease states, initiation, maintenance and propagation of the aberrant cell state. The process of transcription is epigenetically regulated by the orchestrated function of chromatin binding proteins, resulting in accessible DNA and active transcription. This process results in the highly regulated expression of master transcription factors (TFs), effector and supportive housekeeping genes that are required for establishing and propagating cell state. Critically, these processes are dysregulated in neoplastic states by a variety of events, including feedback transcriptional amplification by MYC family TFs (Bradner et al., 2017; Brien et al., 2019). Genome-wide surveys indicate that transcription is globally dysregulated in a wide-array of cancer states, (Grobner et al., 2018; Ma et al., 2018) nominating this process as an important area for therapeutic exploration in cancer.

During the process of transcription, cell-type and context-specific DNA-binding master TFs, coactivators, co-repressors and RNA-binding proteins are necessary to guide the RNA polymerase complex to accessible DNA. Direct targeting of transcription has been possible since the identification of the direct effects of α-amanitin on RNA polymerase (Lindell et al., 1970), however, this compound displays significant toxicities that have limited its use clinically. Other inhibitors with this function, such as triptolide, flavopiridol, and the newer compounds THZ531 and KL1, display improved toxicity profiles, and have in some cases advanced to clinical trials (Table S1). Further, successful compounds directly targeting TFs have been reported. One such example is the small molecule nutlin, which binds to MDM2, displacing p53 from the MDM2 binding site. This indirectly stabilizes p53 by preventing its MDM2-mediated polyubiquitination and proteasomal degradation. Other compounds that similarly target TFs directly by binding to protein-protein interaction interfaces, include stapled peptides targeting p53-MDM2/4 and molecules that stabilize or preclude binding to partner proteins for the TFs MYB, c-MYC, and STAT3 (Table S1). In each case, targeting the TF requires a deep understanding of its interaction domains with binding partners and cognate DNA binding motifs, and is therefore by definition low-throughput. This makes it exceedingly difficult to potently and specifically target individual transcription factors in a high-throughput manner.

As a result of these issues, interest has accrued in the targeting of proteins and cofactors involved in the recruitment of TFs. These targets, including the mammalian SWI/SNF nucleosome remodeling complex, histone writers, readers and erasers, are involved in the

epigenetic programming of cells by establishing sites for transcription, termed the “histone code.” Like dysregulation of transcription, the function of these complexes is similarly dysregulated in neoplastic cell states (Grobner et al., 2018; Ma et al., 2018). These complexes contain enzymes, and therefore are susceptible to inhibitor strategies using active site binding, in addition to allosteric approaches. By targeting these enzymes specifically, generalized and pleiotropic effects on cell growth, survival and apoptosis are realized, mediated by general disruption of transcriptional processes. A key area of concern for inhibitors targeting epigenomic regulators is toxicity due to their propensity for extensive alteration of transcriptional processes genome-wide. Despite this concern, inhibitors of histone methyltransferase, histone deacetylase and lysine demethylases have demonstrated an appropriate therapeutic window, sufficient for clinical translation.

In parallel with the development of potent and specific inhibitors, advances in chemical biology have resulted in a novel strategy, termed *proteolysis-targeted chimera* (**PROTAC**) based degradation (Burslem and Crews, 2020). A PROTAC is a small molecule that binds to both a target protein of interest and an E3 ubiquitin ligase-containing complex receptor, such as CRBN, VHL, or MDM2, resulting in these two protein components being brought physically close together, with subsequent polyubiquitination of the target protein (Fig 1). This leads to proteasomal degradation of the target protein, that may be sufficient to cause near complete target protein loss. Exciting advances using this technology have resulted in the generation of multiple compounds targeting epigenetic regulatory proteins including, among others, the bromodomain and extraterminal domain-containing (BET) proteins. The early BET degrader, dBET1, which utilized N-butyl-2-hydroxyacetamide to link the BET-inhibitor JQ1 with a thalidomide tail, induced highly selective cereblon (CRBN)-dependent BET protein degradation and delayed leukemia progression in mice (Winter et al., 2015). Other BET degraders including ARV825 and MZ1 were simultaneously developed with dBET1 (Table S1). In some cases, these compounds may distinguish between closely related proteins, such as the SJFα and SJFδ compounds that display selectivity for distinct p38 MAP kinase isoforms (Smith et al., 2019). The full potential of PROTACs, enzyme inhibitors and compounds that disrupt protein-protein interaction surfaces to broadly dysregulate epigenetic targets in cancer treatment have yet to be realized, though PROTACs do represent an area of exciting development and a major focus of enquiry for many groups. Exciting advances using this technology have resulted in compounds targeting the Androgen Receptor (AR) in AR+ prostatic carcinoma, and the estrogen receptor (ER) in ER+ breast cancer moving into clinical trials. Examples such indicating the therapeutic potential of PROTACs have fueled increasing interest in PROTAC design and resulted in a variety of new molecules targeting epigenetic and signaling regulators (Burslem and Crews, 2020). Thus, this chemical biology approach represents a novel area of exciting development and a major focus of enquiry for groups interested in targeting transcriptional processes.

Targeting Epigenetic Proteins

Indirect strategies to target proteins that manipulate transcription offer unique opportunities to establish a comprehensive understanding of the transcriptional landscape. Specific post-translational modifications (PTMs) of histone proteins are highly associated with induction and repression of transcription. These modifications, catalyzed by epigenetic writer and

eraser proteins and recognized by epigenetic reader proteins, may be manipulated through enzymatic inhibition or degradation (Fig 2). For clarity, this review will focus on epigenetic regulation of mRNA transcription, though these epigenetic proteins display far-ranging activities on both transcription, epigenetic regulation and indeed, other cellular processes including DNA repair and replication.

Targeting Epigenetic Reader Proteins

Chemical targeting of epigenetic reader proteins was initially demonstrated in two independent studies that reported potent and selective monovalent inhibitors of BET bromodomain family reader proteins— JQ1 and I-BET762 (Filippakopoulos et al., 2010; Nicodeme et al., 2010). In these and subsequent studies, mono or bivalent inhibition of BET bromodomain proteins resulted in striking effects on mRNA transcription. Observations such as these have provided insights into the mechanism of how epigenetic protein reader inhibition manipulates transcription through regulation of key gene transcription, including *c-MYC*, *BCL2* and *CDK6* in different cancer subtypes (Dawson et al., 2011; Delmore et al., 2011). As a result, BET reader inhibition has become the model for a new class of epigenetic-targeted agents, and has stimulated interest in other epigenetic targets for the development of tool compounds and pharmacologic agents.

The bromodomain (BRD) functions as a reader domain for acetylated histones (Owen et al., 2000). In recent years, inhibitors targeting other bromodomain-containing proteins have been developed, such as TRIM24, BRD9, SMARCA4, CBP, and ATAD2 (Table S1). These inhibitors bind to the bromodomains of these individual proteins, and interrupt protein binding to acetylated lysine residues (Kac). Importantly, the effects of these inhibitors on transcriptional regulation is limited, suggesting that interrupted binding of these proteins to acetylated histones alone is insufficient to inhibit the regulatory function of the whole protein. Additionally, using PROTAC technology, bromodomain inhibitors have been used as anchors for degrader development. By this strategy, loss of the full length protein, rather than focal inhibition of the bromodomain is achieved, which targets all functions of these proteins and yields increased effects on transcription. Paralleling inhibitor development, multiple bromodomain based degraders have been recently reported, including those targeting TRIM24, BRD9, and SMARCA4 (Table S1). These compounds, in comparison with parental bromodomain inhibitors, display striking differences in activity, highlighting the differences between bromodomain protein reader function inhibition and protein loss. These newly developed degraders are therefore high priority tools for studying multi-domain protein function beyond the focused inhibition of one single domain.

Histone lysine and arginine methylation are other important and widespread PTMs associated with alteration in chromatin architecture, TF recruitment and transcriptional initiation and elongation. These PTMs may function to either activate or repress transcription (Jambhekar et al., 2019). Lysines can be mono-(me1), di- (me2) or tri- (me3) methylated on their ε-amine group, whereas arginines can be mono-methylated (me1), di-methylated symmetrically (me2s) or asymmetrically (me2a) on their guanidinyl group (Jambhekar et al., 2019). Individual histone methylation reader proteins have preference for

distinct mono, di or trimethylated consensus sequences, imparting specificity to their binding.

Methyl-lysine (Kme) readers comprise more than 200 reader domains clustered in several families including malignant brain tumor (MBT), chromo, Tudor, PWWP and PHD domains (Arrowsmith and Schapira, 2019). The recognition of methyl-arginine (Rme) is predominantly performed by Tudor domains (Sprangers et al., 2003). Drugging Kme reader domains represents a greater challenge compared to KAc reader domains, due to the variability of Kme reader domain volume, enclosure, and binding pocket hydrophobicity (Santiago et al., 2011). Tudor domains frequently occur in tandem arrangements and recognize Kme through canonical aromatic cage pockets, while the isolated Tudor domain reads Rme2s and Rme2a in such a way that the guanidinium group of the arginine is sandwiched in a π-stacking arrangement between two aromatic side chains (Arrowsmith and Schapira, 2019). The K/Rme binding pockets found in Tudor domains are typically small, which pose a challenge for identifying potent ligands targeting the pockets. UNC2170, which binds to dimeric Tudor domains by engaging a larger protein surface at the interface of two p53-binding protein 1 (53BP1) Tudor domains, can compete with H4K20me2 peptides for binding (Perfetti et al., 2015). An alternative and perhaps more promising strategy for targeting tandem Tudor domains may be to disrupt the interface between them (Arrowsmith and Schapira, 2019). Compounds targeting the MBT domain that recognizes Kme1 and Kme2 (Nady et al., 2012) and the chromodomain that recognizes Kme3 have been reported. In the case of chromodomain containing proteins, success has largely been derived using peptidomimetic inhibitors such as UNC3866 (Table S1). Less active, nonpeptidomimetic inhibitors such as MS37452 (Ren et al., 2015) have also been developed and show cellular activity by derepressing the polycomb regulated INK4a/ARF locus at high concentrations. This variability in activity between classical small molecule compounds and peptidomimetics likely reflects the contribution of steric constraints and bonding between compound and the chromodomain. Strategies for targeting other methylation reader domains are still lacking potent and selective molecules that can efficiently dissect the mechanism of these readers.

The Kme binding reader PWWP domain has specificity for H3K36me2/3 or H4K20me residues (Arrowsmith and Schapira, 2019). PWWP domains possess an aromatic cage for Kme binding, flanked by a basic surface that binds DNA (Arrowsmith and Schapira, 2019). BI-9321 is the first potent and selective inhibitor of the PWWP domain, and inhibits the PWWP-domain containing H3K36 methyltransferase, NSD3, resulting in downregulation of *c-MYC* expression in acute myeloid leukemia (AML) cell lines (Böttcher et al., 2019). Chemical interrogation of histone methylation readers is a burgeoning field that is likely to yield key insights, however given the limited progress thus far in generating potent and specific inhibitors, future studies using alternative methodologies such as peptidomimetics, PROTACs and interface binding molecules will be of great interest.

Targeting Epigenetic Writer Proteins

Dynamic and plastic histone modifications created by writer enzymes are responsible for establishing the epigenetic landscape. These marks are regulated by a delicately balanced

crosstalk between readers, writers and erasers that when altered can promote pathogenesis. Here we focus on targeting more transient marks that are deposited by histone writer proteins and their role in transcription, rather than more traditionally heritable marks that may be stable across cell division. Other less well characterized modifications, such as reversible histone serotonylation, crotonylation, glycation, phosphorylation, acylation, and others listed in Fig 2 will not be discussed here due to their limited inhibitor development.

Post-translational acetylation of histones was initially associated with transcription by the experimental finding that the positive charge on acetylated histone tails caused DNA to unravel from histones (Allfrey et al., 1964). This is thought to permit access for TFs and RNA polymerase to bind and promote transcription. While several histone acetylation sites have been thoroughly characterized, the acetylation of histone H3, lysine-27 (H3K27ac) has been extensively studied as a mark of active promoter and enhancer elements that regulate specific gene expression programs (Bradner et al., 2017). As a result, several histone acetyltransferase (HAT) inhibitors have been developed against the HATs that catalyze this mark, aiming to kinetically tease apart the role of this particular acetylation mark on transcription and disease pathogenesis (Dancy and Cole, 2015; Lasko et al., 2017).

Acetylation of H3K27 is performed by the acetylation writer proteins, the E1A Binding Protein P300 (*EP300*) and CREB-Binding Protein (*CREBBP*, *CBP*). Due to their varied roles in the maintenance of cellular homeostasis and proliferation, and their role in pathogenesis of diseases including cancer, these proteins and their function are heavily studied (Dancy and Cole, 2015). In addition to the H3K27ac mark, these proteins also catalyze acetylation of many other histone and non-histone substrates, such as H3K56ac and others (Dancy and Cole, 2015; Weinert et al., 2018). Synthetic chemical probes have been developed that target their catalytic activity, including C646, a pyrazolone and A485, a spiro oxazolidinedione (Table S1). Additionally, inhibitors including CBP30, I-CBP112, and GNE-781, targeting the Kac reader bromodomain function of EP300/CBP were also developed (Table S1). With next-generation, higher potency molecules such as the HAT inhibitor A485, EP300/CBP were recognized as potential therapeutic targets in hematological malignancies and androgen receptor positive prostate cancer (Lasko et al., 2017; Michaelides et al., 2018). However, targeting distinct domains of these proteins appears to have distinct effects. While targeting the EP300/CBP catalytic HAT domain prevents differentiation, targeting the bromodomain promotes differentiation by disrupting cell-type-specific gene expression and cell identity (Ebrahimi et al., 2019). Further, while targeting the bromodomain alone results in loss of H3K27Ac at enhancers, it maintains EP300/CBP occupancy at chromatin. This strategy disrupts enhancer RNA production which is known to suppress EP300/CBP activity (Bose et al., 2017), and, as a result, suppresses the transcription of enhancer regulated genes (Raisner et al., 2018). Intriguingly, targeting both domains simultaneously dramatically delocalizes EP300/CBP from chromatin at promoters to synergistically inhibit prostate cancer cell proliferation (Zucconi et al., 2019). These intriguing studies highlight the distinct functional roles of each domain within EP300 and CBP, and the utility of targeting multidomain proteins using distinct inhibitors targeted against individual domains. Further, the use of a PROTAC agent derived against either domain would obviate the need for selective inhibitors, by degrading the full-length protein.

Although progress has been made in understanding the individual effects of these paralogous proteins on chromatin structure and transcription, little is understood about the distinct roles of each paralog in cancer, in part because developing selective small molecules is challenging with their great structural homology (Dancy and Cole, 2015). Increasing genetic evidence indicates these proteins may be responsible for the control of distinct gene networks (Ramos et al., 2010; Sen et al., 2019). Chemical and mutational strategies to dissect the functions of these large, multi-domain proteins are underway by several groups, and should provide clarification to the roles of these HAT proteins.

In addition to EP300 and CBP, chemical probes targeting P300/CBP-associated factor (PCAF) and the closely related general control nonderepressible 5 (GCN5) have proven promising for the understanding of cancer. PCAF appears to play context-dependent roles as tumor growth repressor or promoter (Jia et al., 2016; Malatesta et al., 2013). With the advent of several potent and selective bromodomain inhibitors including the pyridazinone GSK4027 and triazolopthalazine-based L-Moses (Table S1), the field has moved towards the development of PROTACs to clarify key questions about protein function. After bromodomain inhibition proved unsuccessful, the first PROTAC for PCAF, GSK983/GSK699 was developed (Table S1). Novel inhibitors of other acetyltransferases, including the KAT6 and KAT7 members TIP60, MOZ and HBO1 have been described (Table S1). While studies have helped in clarifying the roles of acetylation on transcription in cancer and other diseases (Baell et al., 2018), much is left to be discovered.

Histone acetylation is opposed, in part, by histone methylation. Histone methylation was thought to be irreversible until the initial discovery of the first demethylase, LSD1/KDM1A (Shi et al., 2004). Methylation has been associated with both promotion and suppression of transcription, enhancer and promoter function and modulation of signaling pathways, and has been reviewed in detail (Jambhekar et al., 2019). Here, we discuss recent findings with key histone methyltransferases and how chemical perturbation has uncovered insights into transcriptional regulation in cancer.

Advancements in small molecule agents against protein lysine methyltransferases (PKMTs) have been developed in the past few years (Morera et al., 2016). EPZ0014777, the first reported inhibitor against the H3K79 histone methyltransferase *DOT1-like Histone Lysine Methyltransferase* (DOT1L), has been used extensively in studies of MLL-rearranged (MLL-r) leukemia (Daigle et al., 2011). Its derivative with optimized pharmacokinetic properties, EPZ5676 (also known as Pinometostat), has advanced into clinical trials in MLL-r leukemias (Morera et al., 2016) and represents a hallmark of the therapeutic potential of targeting PKMTs. The mechanism of EPZ5676 treatment in leukemia cells was narrowed to implicate a functional role for H3K79me2/me3 in chromatin accessibility, acetylation and binding of TFs at H3K79me2/me3-marked enhancers (Godfrey et al., 2019). A separate approach to targeting the MLL complex by targeting the MLL interaction with Menin with small molecules has recently been reported. This results in potent loss of H3K4me3 and inhibition of AML cell growth (Krivtsov et al., 2019). The approach of targeting an interaction domain on MLL to disrupt context-dependent function required significant structural insights, and represents a powerful example for alternatives to classical enzyme inhibition as a mechanism of focused disruption (Krivtsov et al., 2019).

Among the most studied methyltransferases is the catalytic unit of the polycomb repressor complex 2 (PRC2), *Enhancer of Zeste Homolog 2* (EZH2), which has a variety of roles as a master regulator of transcription by catalyzing H3K27me3 (Kim and Roberts, 2016). Using EZH2 inhibitors, such as GSK126, EPZ6438, EPZ011989 and JQEZ5, EZH2's role has been probed in several tumor types (McCabe et al., 2012; Zhang et al., 2016) (Table S1). In diffuse intrinsic pontine glioma (DIPG), which harbors the signature H3K27M mutation resulting in chromatin hyperacetylation, EZH2 activity is still necessary for cell proliferation *in vitro* and *in vivo* (Mohammad et al., 2017; Piunti et al., 2017). EZH2 inhibition targets a proneural subset of glioma stem cells in glioblastoma both in cell culture and *in vivo* (Jin et al., 2017) and similar inhibitors have identified a role for EZH2 in maintaining *MYCN* expression in *MYCN*-amplified neuroblastoma (Chen et al., 2018). Further, EZH2 inhibitors synergize with the HDAC inhibitor panobinostat, indicating a tractable mechanism for inhibition of tumor oncogenes through regulation of multiple histone binding proteins. To this end, targeting EZH2 in combination with BRD4 and MAPK pathway inhibitors is sufficient to overcome epigenetic cross-talk and compensatory mechanisms that promote H3K27ac in EHZ2-aberrant tumors (Huang et al., 2018). These added layers of epigenetic complexity provide mechanisms for overcoming drug resistance and driving antitumor activity through molecular synergy. Several studies have investigated targeting PRC2 component members by generating PROTACs for EZH2 and EED that have effects on EZH2, EED and SUZ12 (Table S1). These valuable additions to the chemical toolbox will be important to dissect the function of the PRC2 complex in cancer and non-neoplastic cells. Recently, the EZH2 inhibitor, Tazverik (tazemetostat, EPZ6438, Epizyme) has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of epithelioid sarcoma, further indicating the translational potential of compounds targeting epigenetic proteins.

In addition to PKMTs, protein arginine methyltransferases (PRMTs) have defined roles in cancer stemness, gene expression, mRNA splicing, and the DNA damage response and therefore represent excellent targets for small molecule development (Jarrold and Davies, 2019). PRMT5 inhibitors like EPZ015666 (GSK3235025), EPZ019997 (GSK3368715), and HLCL-61 have gained momentum due to PRMT5's defined role in a variety of cancers including lymphomas and AML (Table S1). In mantle cell lymphoma (MCL), EPZ015666 treatment prevents SMD3 methylation, resulting in cell death (Chan-Penebre et al., 2015; Tarighat et al., 2016). Similarly, HLCL-61 increased expression of miR-29b, leading to loss of Sp1 and FLT3 expression and AML cell death (Tarighat et al., 2016). In addition, several PRMT4 inhibitors have been recently reported (Table S1). Continued investigation of the role of PRMTs, in different cancer models will be facilitated by an expansion of chemical inhibitors and degraders targeting this intriguing class of epigenetic regulators.

The enzymes that write and erase other less well characterized histone modifications, such as reversible serotonylation, crotonylation, glycation, phosphorylation, and acylation, as listed in Fig. 2 are not discussed further in this review due to their limited inhibitor development, though they remain intriguing concepts to explore through further analysis (Farrelly et al., 2019; Jain and Patel, 2019; Martire et al., 2019; Sabari et al., 2017; Shiio and Eisenman, 2003; Zhang et al., 2019; Zheng et al., 2019).

Targeting Epigenetic Eraser Proteins

Epigenetic modifications are removed by epigenetic “erasers,” including histone deacetylases (HDACs) and lysine demethylases (KDMs) (Li and Seto, 2016; Thinnes et al., 2014). HDACs, in particular, have been excellent targets for drug development, yielding multiple FDA approved drugs, including romidepsin, SAHA, belinostat and panobinostat, for a variety of hematologic malignancies (Romero, 2019) (Table S1). Much like other epigenetic proteins, different cancer subtypes display sensitivity to inhibition or genetic loss of distinct HDACs (Corsello et al., 2017; Gryder et al., 2019b; Tsherniak et al., 2017). The development of a wide array of HDAC targeted inhibitors has led to a chemical toolset to explore the disparate mechanisms of these closely related but distinct proteins in various types of cancer. Recently, we employed a set of epigenetic targeting compounds including the class I HDACs (1,2,3 but not HDAC8) inhibitors Merck60, entinostat, and LW3, to identify that class I HDACs are required for core regulatory transcription in rhabdomyosarcoma cells (Gryder et al., 2019b). HDAC function was required to avoid super-enhancer hyperacetylation, resulting in disruption of three-dimensional chromatin architecture through changes in the charge of phase condensates and loss of RNA polymerase at these sites (Gryder et al., 2019a). These findings are in addition to studies demonstrating that HDAC activity promotes processive transcriptional elongation by both eviction of NELF at promoters (Greer et al., 2015) and release of chromatin bound BRD4 (Hu et al., 2014), providing a potential mechanism for the observation that HDAC inhibitors synergize with BRD4 inhibitors to drive cell death (Fiskus et al., 2014). Several HDAC6-specific degraders have been recently reported, which provides an opportunity to study HDAC6 function by inhibition and degradation in cancer, for example, in melanoma where it promotes tumor growth, and in glioblastoma where it regulates autophagy and migration (Liu et al., 2019; Yang et al., 2018). The use of HDAC inhibitors, however, is complicated by the finding that distinct HDACs may target a variety of overlapping and distinct histone and non-histone substrates *in vitro*. Further, some HDAC inhibitors such as CUDC-907 and romidepsin display effects on both HDACs and the PI3K pathway (Qian et al., 2012; Saijo et al., 2012). One potential solution to this issue would be an unbiased comparative proteomic time-based resolution of HDAC inhibitor function by global acetylome profiling, as has recently been demonstrated for acetyltransferase inhibitors (Weinert et al., 2018).

Histone lysine demethylases (KDMs) regulate the epigenetic landscape in multiple tumor subtypes, including neuroblastoma, AML breast cancer, and Ewing sarcoma (Thinnes et al., 2014). These occur through direct regulation of histone methylsubstrates, including H3K4me1/2 for KDM1, H3K4me3 for KDM5, H3K27me3 for KDM6 and H3K9me2/3 for KDM4 proteins, among others (Zhao and Shilatifard, 2019). While KDM1 proteins use FAD as a cofactor to catalyze demethylation of a substrate, KDM2–7 perform this function using a homologous Jumonji C-terminal domain (JmjC), which uses elemental iron and α-ketoglutarate as cofactors to produce a demethylation reaction (Thinnes et al., 2014). LSD1/KDM1A inhibitors, such as GSK-LSD1, have entered clinical trials (Fu et al., 2017). LSD1 inhibitors, however, display off target toxicities on the hematopoietic system, including thrombocytopenia and neutropenia, which has limited their use (Fang et al., 2019).

Many more KDM2–7 protein inhibitors, including pan and selective inhibitors have been reported, however, unlike GSK-LSD1, their use to interrogate cancer cell growth has been limited by a lack of effective cellular activity or *in vivo* activity. Unique to this group are inhibitors of KDM5, such as CPI-455, that along with inhibiting KDM5, reduce the viability of drug tolerant persister cells (Vinogradova et al., 2016). Of all KDM5 inhibitors, however, compound C49 and its derivatives are the most commonly used chemical probes (Table S1). These inhibitors have been used for targeting transcription in cancer, and with an increasing understanding of KDM5's role in regulating oncogenesis, its validity as a target for therapeutic intervention has only increased. Using KDM5 inhibitors, roles have been identified for the protein in driving lineage-specific cancer cell growth and chemoresistance (Paroni et al., 2019). Similarly, KDM6 inhibitors, such as GSK-J4 that targets KDM6A (UTX) and KDM6B (JMJD3), displays preclinical activity in multiple tumor models (Table S1) (Hong et al., 2019; Lochmann et al., 2018). Although targeting KDMs by small molecule inhibition has been shown to have important effects, there has been relatively limited development of compounds targeting KDM proteins outside of inhibitors. This suggests alternative methodologies, such as peptidomimetics and degraders, as a potential avenue of interest.

Targeting Nucleosome Remodeling Factors

A critical early step in the establishment of DNA that is accessible to TFs and epigenetic modifying enzymes depends on the activity of ATP-dependent chromatin remodeling complexes that act to pack and unpack chromatin structure. These complexes, including the SWI/SNF (BAF), ISWI, NuRD/Mi-2/CHD, INO80 complexes, modulate chromatin by removing, translocating and restructuring the nucleosome, such that DNA becomes accessible to proteins including TFs, co-activator proteins and the RNA polymerase complex (Wang et al., 2007). In addition to nucleosome remodeling, the ISWI and BAF complexes also mediate binding of CTCF and CoREST complexes to the three-dimensional chromatin architecture (Aitken et al., 2018; Inoue et al., 2019), making them key targets as these processes are dysfunctional in cancer. This has resulted in increased attention on the BAF complex in recent years, fueling a deeper understanding of structure, and therefore, increased potential for the development of inhibitors.

Mammalian BAF is a multisubunit enzyme containing complex including helicases and ATPases, along with subunits that coordinate complex formation. Distinct BAF complexes display different subunit compositions, resulting in an opportunity to target different complexes through targeted chemical inhibition of specific subunits. The BAF core complexes contain SMARCA2 and SMARCA4, homologous proteins with both ATPase activity and bromodomains. The development of the potent and selective SMARCA4 bromodomain inhibitor PFI-3 (Fedorov et al., 2015) identified that the bromodomain of SMARCA2/4 is not essential for BAF complex function in these cells (Vangamudi et al., 2015). On the other hand, a recently reported inhibitor, compound 14, blocks the ATPase catalytic function of SMARCA2/4, resulting in broad and striking effects on cancer cell growth (Papillon et al., 2018). These data emphasize the relative importance of the ATPase domain to BAF complex function. Recently, a PROTAC directed toward the bromodomain of SMARCA4, ACBI1, was developed that has potent effects on SMARCA2/4-dependent

leukemia cell lines and no effects on SMARCA2/4-deficient cell lines, indicating on-target activity (Farnaby et al., 2019).

Independent of targeting the catalytic units of the BAF complex, newer compounds such as the 12-membered macrolactam compounds like BRD-K98645985 (BD-98) are useful for binding ARID1A-containing BAF complexes, resulting in physical displacement of these complexes from chromatin in ES cells and HCT116 colorectal carcinoma cells (Chory et al., 2019; Marian et al., 2018). Further examination of the effects of BD-98 on ARID1A-mediated chromatin interactions and BAF complex assembly are necessary to fully decipher its mechanism of action. These findings have provided deep insights into BAF complex function through interrogation of individual complex members, and sparked significant interest in further exploration.

In addition to ARID1A and SMARCA2/4, BRD7 and BRD9 of the BAF complex play crucial roles in cancer. Recently, selective BRD9 bromodomain inhibitors have been reported, including BI-9564, and I-BRD9 (Table S1). In AML cells, selective inhibition of BRD9 caused suppression of *c-MYC* transcription, cell cycle arrest and differentiation, with limited effects on other BAF dependent cell lines (Hohmann et al., 2016). The BRD9 inhibitor BI-9564 was further used as the substrate for a PROTAC, dBRD9. Though it has promising activity, the antitumor effect of dBRD9 is not as severe as SMARCA4 knockout (Brien et al., 2018a), highlighting the key role of the ATPase function of BAF complexes. Despite these findings, synovial sarcoma and malignant rhabdoid tumor cell lines that are characterized by dysfunctional BAF complexes are uniquely sensitive to loss of BRD9, through either genetic means or selective degradation with dBRD9 (Michel et al., 2018). These data suggest that alterations in BAF complex structure may yield unique dependency on non-catalytic subunits. Independently, malignant rhabdoid tumor cell lines were demonstrated to be sensitive to the combined BRD7 and BRD9 degrader VZ185, further supporting these findings (Zoppi et al., 2019). With the recent cryo-EM structure of nucleosome-bound BAF complexes being solved, deep mechanistic insights into the results of inhibition or degradation of specific subunits are likely to be identified (He et al., 2020).

Combination and Chimaeric Inhibitors

One key issue with compounds targeting epigenetic proteins, however, remains the rapidity over which they act -often requiring several cell cycles in order to elicit their maximal effects. Thus, interest has turned to combination of compounds to elicit stronger and more rapid effects. In addition to combination of agents, an alternative approach is to develop inhibitors that target multiple proteins with a single molecule. To this end, one bifunctional molecule has been reported termed Corin, that targets both LSD1 and HDAC1 simultaneously (Anastas et al., 2019; Kalin et al., 2018) (Table S1). This molecule blocks the function of two members of the CoREST complex, and has striking effects in models of glioma and melanoma. While preliminary, this strategy represents a novel and potentially useful method for inhibiting multiple subunits within a single complex, or in addition, between complexes, each of which may be selectively required for tumor cell growth.

Conclusions and Future Directions

Exciting advances remain on the horizon for the generation of compounds targeting epigenetic readers, writers and erasers. Along with the central role of epigenetic proteins in regulating cell state, differentiation and growth has been the recognition that they are often dysregulated in disease states, and therefore act as key mediators of tumor cell growth, survival and apoptosis. In parallel, by harnessing structural information to elicit focused efforts by many groups to derive targeted chemical inhibitors with selectivity for one member of a protein class over all others, there is now a preliminary “toolbox” of compounds for use in a variety of tumor states. The power of genome-scale functional genomics, coupled with new chemical methodologies, including structural-based peptidomimetics and PROTAC technology, has permitted rapid advances in pharmacological modeling of knockout methods, in addition to inhibition, and this has yielded deep insights into the differential importance of full length protein products, compared to inhibition of specific domains.

Despite these advances, several key tools remain missing and are priorities for development. For many described classes of epigenetic enzymes, even tool compounds for selective inhibition are currently lacking. Large-scale, non-profit groups focused on targeting these epigenetic enzymes with open-source chemical probes, such as the Structural Genomics Consortium and Chemical Probe Portal (Muller et al., 2018), have set the tone for appropriate resource sharing. With the rising use of functional genomics to define the genes required for tumor-specific cell growth, we are poised to define important targets for inhibition or degradation. Thus, key future directions include focused derivation of a toolbox of compounds to the targets most highly relevant to individual tumor states, which may include MYC family oncogenes, multiple subunits of key regulatory complexes such as the BAF complex in synovial sarcoma or the MLL complex in leukemia, in addition to the aberrant transcriptional complexes formed by chimaeric fusion oncoproteins, such as PAX3-FOXO1, EWS-FLI1, TMPRSS2-ERG, ETV6-NTRK3 in rhabdomyosarcoma, Ewing sarcoma, prostatic carcinoma and infantile fibrosarcoma, among many others. Finally, as described, the role of novel regulators yet to be defined that perform only recently described histone modifications including serotonylation, crotonylation, lactylation, phosphorylation and ubiquitination/SUMOylation remain to be explored, and chemical targeting of these modifications will be a useful adjunct to genetic studies to identify the role of these and other future epigenetic post-translational modifications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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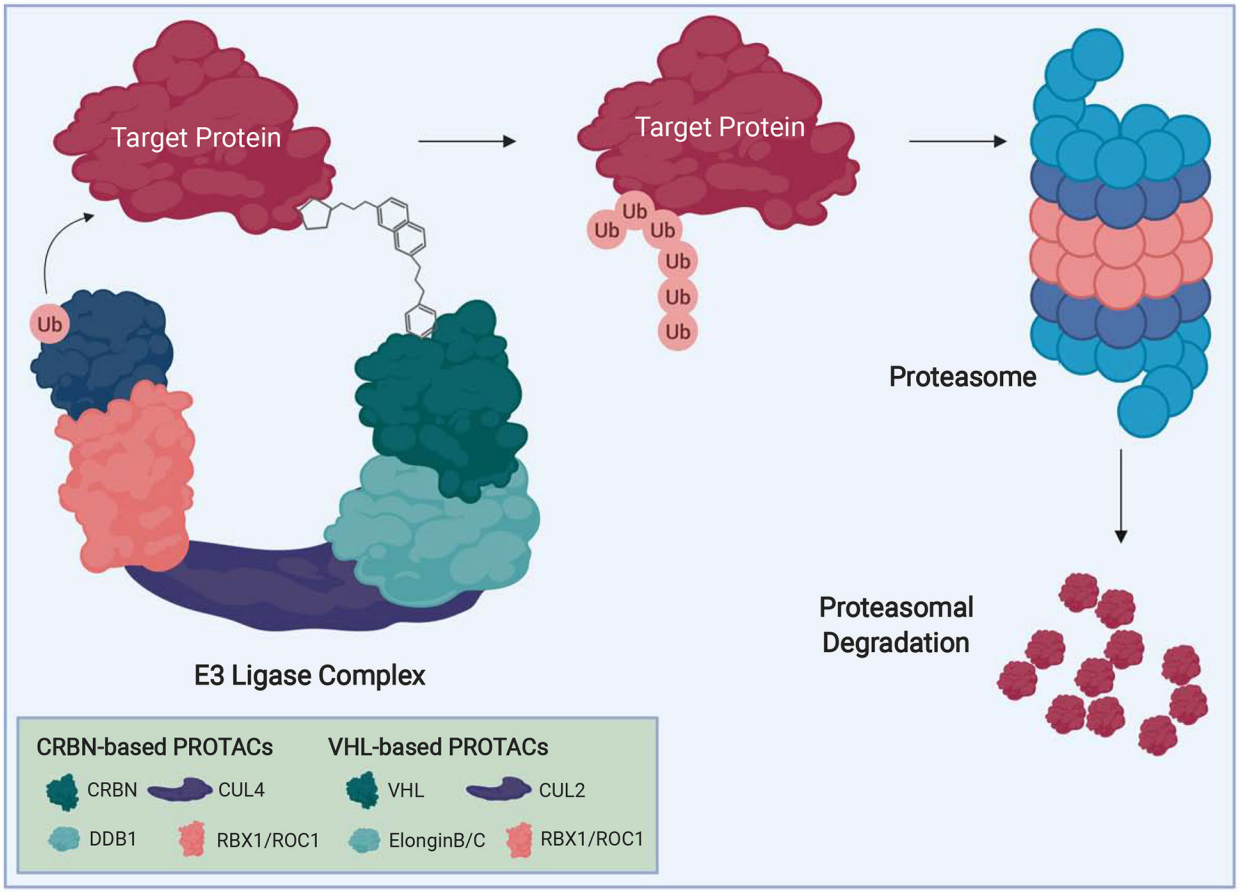

Figure 1. PROTACs bridge a target protein of interest and an E3 ligase complex to induce selective degradation.

By the PROTAC strategy, a chemical structure bridging a target protein of interest (red) and the substrate receptor of an E3 ubiquitin ligase containing complex (green) results in E2-dependent ubiquitination of the target protein. This leads to recruitment of the target protein to the proteasome and proteasomal degradation. Differences between CRBN and VHL-based PROTAC complexes are demonstrated.

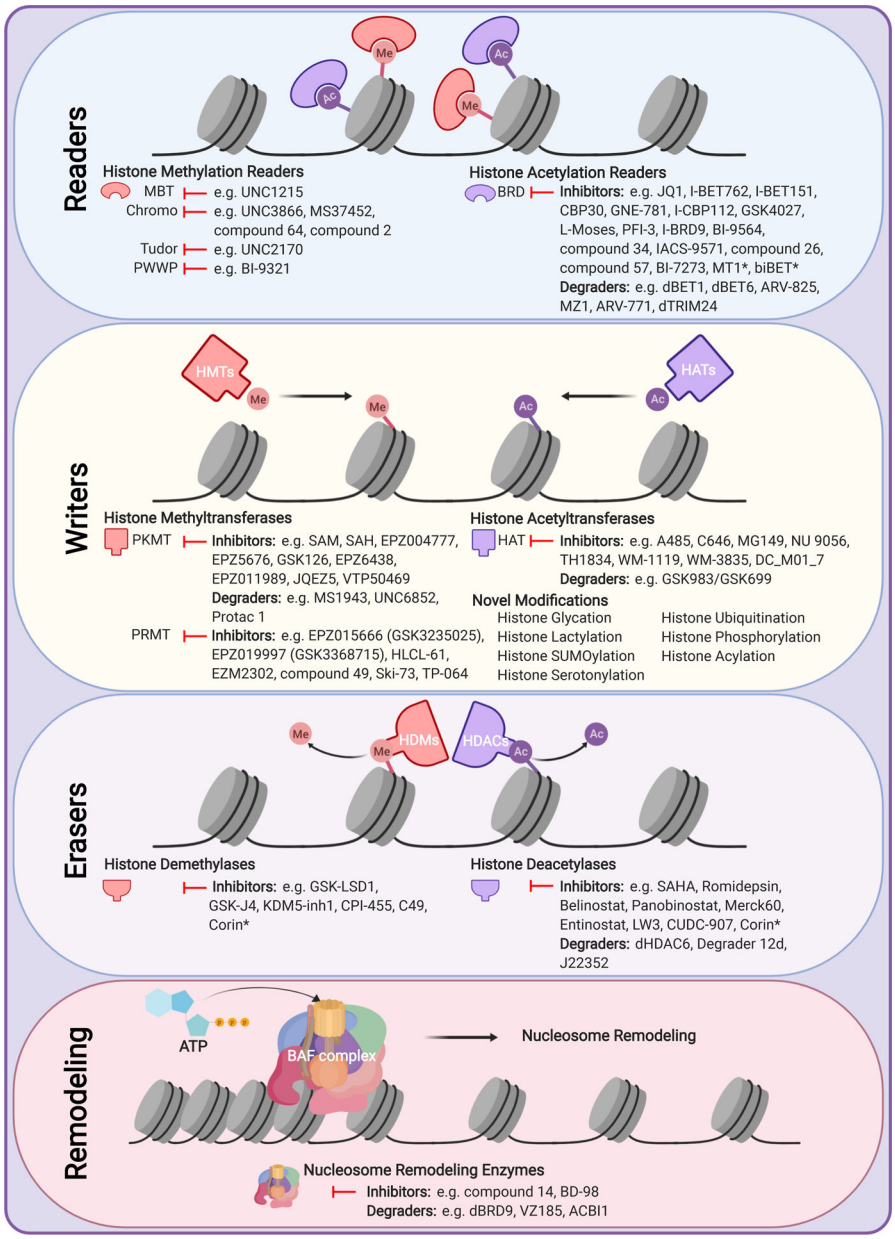

Figure 2. Epigenetic proteins drive alterations that can be targeted using chemical inhibitors and degraders.

Demonstrated are common classes of histone readers, writers and erasers, as well as nucleosome remodeling complexes, and their different classes of inhibitors. MBT - malignant brain tumor; PWWP - Proline-Tryptophan-Tryptophan-Proline; PKMT - protein lysine methyltransferase; PRMT - protein arginine methyltransferase; BRD - bromodomain; HAT - histone acetyltransferase; HDM - histone demethylase; HDAC - histone eacetylase; * - bifunctional inhibitor.