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Pharmacological modulation of transcriptional co-regulators in cancer

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Abstract

Upon binding of transcription factors to *cis*-regulatory DNA sequences, transcriptional co-regulators are required for activation or suppression of chromatin-dependent transcriptional signaling. These co-regulators are frequently implicated in oncogenesis via causal roles in dysregulated, malignant transcriptional control and have represented one of the fastest growing target classes in small-molecule drug discovery. However, challenges in targeting co-regulators include identifying evidence of a cancer-specific genetic dependency, matching the pharmacologically addressable protein fold to a functional role in disease pathology, and achieving the necessary selectivity to exploit a given genetic dependency. Here, we discuss how recent trends in cancer pharmacology have confronted these challenges, positioning co-regulators as tractable targets towards the development of emergent cancer therapies.

Keywords

cancer; transcription; chromatin; therapeutics

Transcriptional Biology and Cancer Pharmacology

Proper spatiotemporal regulation of lineage-specific transcriptional processes is essential for normal organismal development, tissue function, and homeostasis. In a malignant cell state, mutationally altered genomes are accompanied by widespread dysregulation of transcriptional programs that ultimately promote self-renewal and unrestricted proliferation – as recently reviewed [1]. Knowledge of this type has motivated academic and industrial efforts to selectively alter transcriptional regulators with small-molecule tools and drugs. Unfortunately, with the exception of nuclear hormone receptors and limited other examples, a lack of ligandable (see Glossary) sites on transcription factors has made them impractical

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targets for direct-acting inhibitors. Instead, efforts to target the co-regulators required for transcription factor signaling have emerged as a viable alternative strategy.

Co-regulatory activity is encoded into diverse functional classes of proteins, including, but not limited to, chromatin modifiers (writers and erasers), chromatin remodelers, chromatin readers, and other scaffolding proteins (Figure 1). Chromatin modifiers are enzymes that function to post-translationally modify histone proteins, which alters the recruitment of chromatin readers that bind to modified histone substrates and can also alter the biophysical interaction between histones and DNA [2]. Chromatin remodelers leverage ATP hydrolysis to catalytically alter nucleosome spacing and composition, affecting the accessibility of the underlying genome [3]; some also possess the ability to evict chromatin-bound complexes that possess antagonistic regulatory function [4]. Many co-regulators do not act enzymatically on a nucleosome substrate, but instead they serve as scaffolds to assemble diverse regulatory complexes at **cis-regulatory elements**. As with all cancer therapy, the goal of targeting co-regulators is to elicit tumor-specific cell killing in a manner that maintains the survival and physiology of normal cells in the body. Thus, the importance of high-quality target identification and validation studies – whether accomplished by genetic experimentation or by use of tool compounds – cannot be overstated (as recently highlighted [5]). Ultimately, pharmacologic development of a given target is poised to succeed when: (i) the target protein is a **cancer-specific dependency**, (ii) the cancer-relevant function of the protein is addressable by pharmacologic approaches, and (iii) sufficient selectivity can be achieved by a pharmacologic agent to exploit the vulnerability of interest without eliciting toxic off-target effects.

Conceptually, a transcriptional co-regulator, like any protein, can be considered a cancer-specific dependency through two genetic mechanisms: oncogene [6] and non-oncogene [7] addiction (Box 1). For the practice of drug discovery, cancer-specific dependencies provide a genetic rationale for pharmacologically modulating a target with the prospect of achieving a high therapeutic index. In the following, we review recent advances in the pharmacology of transcriptional co-regulators, attempting to highlight each target in the context of its role as an oncogene or non-oncogene dependency in human cancers. We begin with targets regulating transcriptional elongation, discuss diverse bromodomain-containing cancer targets, and finish with a highlight of emerging targets involved in histone methylation.

Targeting RNA Polymerase II Pause Release

CDK9

After transcriptional initiation, RNA Polymerase (Pol) II is subjected to a tightly regulated pausing step just downstream of transcription start sites. From this promoter-proximal pausing, Pol II awaits molecular licensing for productive transcriptional elongation via the kinase activity of positive transcription elongation factor b (P-TEFb), a heterodimeric complex of cyclin-dependent kinase 9 (CDK9) and Cyclin T. P-TEFb is inhibited by the promiscuous CDK inhibitor, flavopiridol, and thus, CDK9 pharmacology has been extensively studied in human cancers [8].

Despite positive anti-cancer efficacy, CDK inhibitors have historically been limited clinically by off-target pharmacology and dose-limiting toxicity. However, pharmacologic advances enabling selective perturbation of CDK9 have prompted a reconsideration of CDK9 inhibition as cancer therapy. Recently disclosed agents include the inhibitors, iCDK9 [9], NVP-2 [10], and MC180295 [11], and a selective degrader, THAL-SNS-032 (Figure 2, Key Figure) [12]. As selective CDK9 agents enter the clinic, it will be instructive to observe how gains in selectivity affect the therapeutic index of perturbing CDK9 in patients. Notwithstanding the notable advances in pharmacological development, the intrinsic target biology of CDK9 may yet provide challenges for drug development; namely, that CDK9 kinase activity is a universal requirement for productive gene transcription [12-15]. It follows that on-target, dose-limiting toxicity may limit the therapeutic index of CDK9 inhibition as healthy tissues may be equally affected by global loss of transcriptional activity. Careful dosing regimens may help mitigate on-target toxicities, but the anticipation of this issue has motivated efforts to inhibit transcriptional co-activators with more specialized roles across the genome.

BET Family Proteins

Bromodomain protein 4 (BRD4) is a transcriptional co-activator known to associate with CDK9 and affect pause release of RNA Pol II. BRD4 is part of the bromodomain and extra-terminal (BET) family of proteins (BRD2, BRD3, BRD4, and BRDT), each of which possess a pair of tandem bromodomains positioned at their N-terminus. Bromodomains are protein folds evolutionarily adapted to bind to acetylated lysine side chains found within histones and on other chromatin-bound proteins and this function positions BRD4 at transcriptionally active *cis*-regulatory elements. Because of its links to CDK9 activity, BRD4 has emerged as a compelling target to pharmacologically disrupt pause release at select target genes, rather than across the entire genome.

BRD4 was first causally linked to cancer pathogenesis by the discovery that chimeric fusions of BRD4 to NUT (nuclear protein in testes) form oncogenes that drive a severe squamous cancer termed NUT midline carcinoma (NMC). Early efforts with JQ1 (Figure 2), a first-in-class bromodomain inhibitor, established BET bromodomain inhibition as a promising strategy to target BRD4 oncogene addiction [16], which has inspired ongoing clinical testing in NMC [17,18]. In these tumors, BET bromodomain inhibition targets BRD4-NUT oncogene dependency by displacing the fusion oncoprotein from chromatin, ultimately leading to squamous differentiation and arrested proliferation [16].

Interestingly, wild-type BRD4 has also been identified as a non-oncogene addiction in a variety of human cancers. Hematological malignancies, the first tumors shown to require wild-type BRD4, are hypersensitive to BET bromodomain inhibition [16,19-22], which has motivated a number of clinical trials that have reported preliminary signs of efficacy [23-25]. While response rates in early Phase 1 reports have not been as high as hoped based on preclinical studies, side-effects have been relatively mild – the most common being thrombocytopenia – suggesting BET bromodomain inhibition may be most compellingly used as part of combination therapies. Nevertheless, lower-than-expected response rates also

highlight our need to discover predictive biomarkers of sensitivity so that drug candidates can be better matched to the patients most likely to receive benefit.

While predictive biomarkers remain elusive, the mechanisms of sensitivity to BET bromodomain inhibition in BRD4-dependent tumors have been extraordinarily well studied. In comparison to CDK9 inhibition, BET bromodomain inhibitors elicit relatively narrow transcriptional responses, preferentially affecting a class of genes driven by large enhancer clusters – often referred to as super-enhancers – that host massive amounts of chromatin-bound BRD4 [26]. This class is enriched for cell-type identity genes and in cancer, this includes many proto-oncogenes [26,27]. Thus, the mechanism of BET bromodomain inhibitor sensitivity is typically attributed to preferential downregulation of tumorigenic transcription factors, perhaps best exemplified by *MYC* suppression in hematological malignancies [19,20].

Recently, BET proteins have been at the center of breakthroughs in the field of targeted protein degradation. Proteolysis targeting chimeras (PROTACs) are bi-functional small molecules that recruit a liganded target protein to an E3 ubiquitin ligase complex to induce proximity-mediated poly-ubiquitination and proteolysis (Figure 3A) [28]. The recent use of small-molecule immunomodulatory drugs (IMiDs) as the E3-targeting moiety has allowed for targeted protein degradation *in vivo* and brought renewed interest in small-molecule degraders as therapeutics and chemical tools [29]. This was first demonstrated using dBET1, a phthalimide conjugation of JQ1 that simultaneously binds cereblon (CRBN) and BET proteins to induce CRL4^{CRBN}- dependent degradation of the BET family [29].

Our work with dBET6 (Figure 2), a BET degrader optimized for cellular permeability, has revealed major differences between the cellular responses to BET degradation and BET bromodomain inhibition (Figure 3B). In contrast to the selective transcriptional effects evoked by BET bromodomain inhibition, BET degradation results in complete collapse of mRNA production by preventing the release of RNA Pol II from promoter proximal pausing genome-wide [15]. It had previously been suggested that BRD4 recruits CDK9 to chromatin and that this was the mechanism by which BRD4 affects RNA Pol II pause release [30]. Quite interestingly, while CDK9 activity is indeed eliminated by BET degradation, it does not result in loss of CDK9 occupancy on chromatin [15]. Recent application of the **auxin-inducible degron** system [31] to selectively degrade BRD4 has elaborated on these results, demonstrating that like BET degradation, BRD4 degradation results in transcriptional collapse without affecting CDK9 occupancy [32]. Thus, the mechanism by which BRD4 regulates CDK9 activity remains an area of open investigation.

While the BET bromodomain inhibitors and BET degraders mentioned above target the entire BET family, degradation tools have recently enabled BRD4-specific pharmacological perturbations. Remarkably, attempts to optimize BET-targeted PROTACs to only degrade BRD4 have been successful, resulting in the disclosure of multiple BRD4 degraders that do not affect BRD2 or BRD3 protein levels [33-35]. We are hopeful that PROTAC development will eventually provide chemical tools to study all BET proteins individually, each of which may have unique roles in cancer pathogenesis. For example, genetic depletion of BRD2, which can act as an oncogene to drive B-cell malignancies [36], also diminishes growth of

BRD4-dependent breast cancer and chronic lymphocytic leukemia cells [37,38], but not AML cells [39]. This may reflect the fact that BRD2 and BRD4 have at least partially non-redundant function, with BRD2 functioning in three-dimensional genome organization [40,41], and highlights the need for chemical probes to disentangle BET family target biology. Still, BET degradation has already proved a very useful tool for studying BRD4-dependent transcriptional regulation in cancer cells.

Given concerns about global inhibition of mRNA synthesis causing on-target toxicity in normal tissues, BET degraders will potentially face the same set of challenges encountered by CDK9 inhibitors in the clinic. Nevertheless, disclosures of BET degrader activity *in vivo* have been promising [15,29,42-44], notably including a picomolar-potent molecule, QCA570, which shows anti-tumor activity in mice at 1 mg/kg dosing [44]. From a therapeutic perspective, a particularly intriguing use for pan-BET or BRD4-specific degraders would be as a means to overcome BET bromodomain inhibitor resistance. In each of three index reports, resistance to BET bromodomain inhibition was attributed to rewiring of transcriptional signaling pathways in a manner that dispensed of BET bromodomain requirement [37,39,45]. In leukemia, resistant cells are able to sustain or rapidly reactivate *MYC* expression upon BET bromodomain inhibition due to increases in Wingless and Int-related (WNT) signaling [39,45] – confirming the existence of BET-bromodomain-independent mechanisms of maintaining *MYC* expression. However, in both leukemia and breast cancer models of evasive resistance, genetic addiction to BRD4 is retained, suggesting that BET degradation, which is able to recapitulate genetic depletion of BRD4, might be able to overcome bromodomain inhibitor resistance. While this has not yet been tested, it may offer a compelling rationale for moving BET degraders into clinical trials.

ENL YEATS

ENL (eleven nineteen leukemia) is a chromatin reader and transcriptional co-activator that regulates pause release as a member of the super elongation complex (SEC) [46], a higher-order P-TEFb-containing complex [47]. It possesses an N-terminal YEATS domain, which binds to acyl-lysine side chains on histone H3, including several longer short-chain fatty acid groups (e.g. crotonyl-lysine) that bromodomains do not bind [48-50]. By means of CRISPR-Cas9 loss-of-function screening, we and others identified ENL as a non-oncogene dependency in acute leukemia [46,51]. While ENL is required for the survival of acute leukemia cells *in vivo*, normal hematopoietic stem and progenitors cells are insensitive to loss of ENL, suggesting ENL-targeted therapeutics might be able to elicit cancer-specific cell killing with a wide therapeutic window [46,51].

Recognizing ENL as a potential therapeutic target, we sought to identify a function within the ENL protein that would be actionable for drug discovery. Especially in multidomain proteins, it is not always obvious whether a specific domain within a potential drug target will contribute to the pathogenic function of that protein [52,53]. Thus, we and others used a mutagenesis approach to demonstrate that the YEATS domain is essential for leukemia cell survival by mediating the localization of ENL to acetylated chromatin structures [46,51]. Building on this, chemical discovery efforts have already produced small-molecule

inhibitors of ENL YEATS, including SGC-iMLLT (Figure 2) [54], though further work is required to evaluate the biological activity of these molecules.

Interested in the transcriptional mechanisms underlying ENL sensitivity, we sought to study ENL by a pharmacological approach. To that end, we developed a chemically inducible protein degradation system, **dTAG**, to model an acute pharmacologic perturbation of ENL [46]. The rapid kinetics of this system permit evaluation of direct mechanisms of protein function, which we leveraged to study ENL-dependent transcription in leukemia [46,55]. Coupling acute degradation of ENL to integrative transcriptional genomics, we identified a class of target genes – highly enriched for undruggable leukemic driver genes, including *MYC* and *MYB* – that are selectively downregulated by loss of ENL [46]. Compared to others, these genes feature disproportionately high loads of promoter-bound ENL and upon ENL degradation, they are preferentially depleted of promoter-bound SEC before being selectively downregulated [46]. Thus, ENL selectively drives a tumorigenic gene expression program by recruiting the SEC to leukemic driver genes. Since co-regulators are often involved in rapid induction or suppression of transcriptional activity, it is often difficult to differentiate between primary mechanisms of action and secondary transcriptional effects with the use of genetic perturbations. However, modeling pharmacologic perturbations with systems like dTAG, as done with ENL, offers the ability to resolve phenotypes at earlier time points, aiding in both mechanistic basic biology and translational target validation studies [56].

These mechanistic data highlight an interesting relationship between ENL and BRD4. The phenotypic effects of ENL degradation and BET bromodomain inhibition are similar in that they both affect promoter proximal pause release and preferentially suppress expression of tumorigenic transcription factors. However, BET bromodomain inhibition affects a much larger fraction of the transcriptome, suggesting that they function distinctly. In agreement with this, combining ENL loss with BET bromodomain inhibition shows additive effects on leukemia proliferation *in vivo* [51]. An interesting hypothesis for their discrete regulatory roles would be that ENL and other proteins regulate P-TEFb recruitment while BRD4 controls its activity once it is placed onto chromatin.

Co-Regulators with Non-BET Bromodomains

The early success of BET bromodomain inhibition illustrated the feasibility of targeting acetyl-lysine recognition by chromatin readers and in turn, inspired a wave of efforts to target other, non-BET bromodomains. These ongoing efforts have been extraordinarily successful, ultimately drugging nearly 2 dozen different bromodomain targets [57]. Since not all of these proteins are applicable to cancer, we will focus on a handful of especially instructive examples, which highlight the complex manner by which bromodomains can contribute to protein function and affect – or not – cancer cell survival.

SWI/SNF complex – SMARCA2/4

The SWI/SNF, or BAF, complex (Box 2) is a large, multisubunit, ATP-dependent chromatin remodeler complex that functions at cis-regulatory elements to maintain accessible chromatin structures. Its ATPase function is encoded in two mutually exclusive and highly

homologous proteins: SMARCA2 and SMARCA4. Mutations to *SMARCA4*, which frequently occur in non-small-cell lung carcinomas (NSCLC), are synthetic lethal with loss of SMARCA2, which is otherwise non-essential for cell survival [58-60]. In addition to their highly homologous ATPase domains, SMARCA2 and SMARCA4 also possess highly homologous bromodomains, which have been successfully targeted by a selective, cell-permeable small molecule, PFI-3 [61,62]. Unfortunately, this compound is unable to displace SMARCA2/4 from chromatin and does not recapitulate the anti-proliferative effects of genetic knockdown [52]. Ultimately, functional genetic experiments have revealed that the catalytic ATPase domain, but not the bromodomain, of SMARCA2 is essential in the context of SMARCA2 dependency and is thus the relevant drug target [52]. It is interesting to note that the dispensability of the SMARCA2/4 bromodomains might have been foreshadowed by work in *Drosophila melanogaster* defining the bromodomain of the fly homolog, BRM (Brahma), as non-essential for localization of the complex to active chromatin [63]. Nevertheless, a recent breakthrough in discovery chemistry has produced the first pharmacologic inhibitors of SMARCA2/4 ATPase activity: potent diheteroaryl ureas (Figure 2, Compound 14) that act via allostery and diminish tumor growth *in vivo* upon oral dosing [64]. While in-depth biological characterization has yet to be reported with this series of molecules, they are positioned to advance SMARCA2/4 pharmacology in therapeutically relevant ways. Importantly, these molecules establish the feasibility of targeting chromatin remodeler ATPase domains with drug-like small molecules and we are hopeful that their discovery will buoy increased enthusiasm for chromatin remodeler discovery chemistry. However, despite demonstrating an ability to target the relevant ATPase function, the selectivity needed to realize the promise of SMARCA2 as a synthetic lethality remains a significant hurdle to overcome, urging discovery of additional targets for pharmacological development.

SWI/SNF complex – BRD9

BRD9, which is a member of the non-canonical SWI/SNF complex (ncBAF) [65,66], represents another target for therapeutic disruption of non-oncogene dependencies present within the SWI/SNF complex. A recent study has demonstrated that BRD9, and specifically its bromodomain, is required for the growth of acute myeloid leukemia (AML) cells through the maintenance of high *MYC* expression [67]. This was confirmed using the chemical tool BI-7273, a potent inhibitor of BRD7 and BRD9 [67]. Like BET bromodomain inhibitors, BRD7/9 inhibitors have also been converted into PROTACs via conjugation to E3-binding small molecules [68,69]. dBRD9 (Figure 2), a PROTAC built via IMiD conjugation of BI-7273 is a potent and selective BRD9 degrader, which remarkably, does not degrade BRD7 [68]. Already, this tool has been put to use in contemporaneous studies that discovered a synthetic lethal dependency on BRD9 in BAF-mutant tumors [65,70]. Together, these studies identified that sarcomas and rhabdoid tumors that are driven by mutations to BAF components are highly sensitive to loss of BRD9 via genetic disruption or exposure to dBRD9 [65,70].

CBP/P300

CREB binding protein (CBP) and p300 (adenovirus E1a associated 300 kDa protein) are two closely related histone acetyltransferases (HATs) that act as transcriptional co-activators by

catalyzing the acylation of histone and non-histone lysine side chains (including H3K27ac, a modification found at promoters and putatively active enhancers). Owing to their synthetic lethal relationship, CBP and p300 have emerged as highly attractive cancer targets, particularly in certain lung cancers and lymphomas that frequently harbor mutations to these proteins [71-73]. In addition to their HAT domains, CBP and p300 also feature highly homologous bromodomains, the pharmacology of which has rapidly matured in recent years to include several potent and selective inhibitors [74-78]. Impressively, the successful development of CBP/p300 bromodomain inhibitors has been followed quickly by the disclosure of the first chemical probe for CBP/p300 HAT activity, an indane spirooxazolidinedione, A-485 (Figure 2) [79,80]. A-485 represents a major leap forward in HAT pharmacology, as previous agents were troubled by off-target activity, electrophilicity, and a tendency toward aggregation [81]. While none of the presently existing small-molecule inhibitors of CBP/p300 feature sufficient selectivity to exploit synthetic lethality between the two proteins, convergent chemical biology from separate groups using either A-485 or the CBP/p300 bromodomain inhibitor, GNE-272 (Figure 2), have revealed a selective dependency on CBP/p300 in androgen receptor-positive, but not androgen receptor-negative, prostate cancer growth [79,82]. Perhaps unexpectedly, these molecules are not promiscuously cytotoxic, but show context-specific anti-cancer effects, both within and among different cancer lineages and subtypes. From both a therapeutic and basic biological perspective, it will be important to identify predictive biomarkers of sensitivity and mechanisms of transcriptional response to loss of CBP/p300-mediated lysine acetylation. However, these molecules have already revealed fascinating target biology that would have been difficult to discover without pharmacologic tools. Notably, CBP/p300 bromodomain inhibition has revealed that while the bromodomain is not required for chromatin localization, it is essential for CBP/p300-mediated H3K27 acetylation and enhancer activity [83]. Moreover, CBP/p300 HAT inhibition coupled to quantitative proteomics have revealed that the repertoire of CBP/p300 substrates vastly exceeds a few histone and non-histone substrates; instead, CBP/p300 function through a so-called “acetyl-spray” mechanism, modifying thousands of sites at *cis*- regulatory elements [84]. Very importantly, the discovery of A-485 has definitively established that HATs can be potently and selectively inhibited by drug-like small molecules, now further confirmed by the recent disclosure of inhibitors for the structurally distinct MYST-family acetyltransferases, KAT6A and KAT6B [85]. We expect these discoveries to inspire increased consideration of HAT pharmacology for both chemical biology and cancer therapy.

The above considerations of bromodomain pharmacology offer instructive examples of the diverse outcomes that are possible when binding drug-like small molecules to a chromatin reader domain. While BET bromodomain inhibitors displace BET proteins from chromatin, CBP/p300 bromodomain inhibitors allosterically suppress HAT activity and SMARCA2/4 bromodomain ligands are entirely ineffectual. Using the co-repressor, TRIM24, as an example below, we will discuss how targeted protein degradation can rescue ineffectual ligands, creating high-quality chemical probes that phenocopy genetic depletion.

TRIM24

TRIM24 is a member of the TRIM/RBCC protein family, consisting of an N-terminal tripartite motif and a C-terminal tandem plant homeodomain finger-bromodomain, and has been implicated as a non-oncogene dependency in breast and castrate-resistant prostate cancer [86,87]. Previously, two groups independently disclosed dimethylbenzimidazolone scaffolds as dual inhibitors of TRIM24 and BRPF1 bromodomains, including IACS-9571 [88,89]. Despite double-digit nanomolar affinity, cellular pharmacology with IACS-9571 has been modest [90], inspiring PROTAC development via IMiD conjugation, which yielded a selective degrader of TRIM24, dTRIM24 [91]. Compared to IACS-9571, dTRIM24 elicits increased anti-proliferative effects in AML cells, which are preferentially sensitive to RNAi depletion of TRIM24 [91]. This increase in active pharmacology is rationalized by the discovery of an AML-specific dependency on the TRIM24 **RING domain** [91], which is not addressed by TRIM24 bromodomain inhibitors. Like SMARCA2/4, it seems that the bromodomain is not the relevant function of TRIM24 with respect to its role in AML, which highlights the importance of identifying domain-specific dependencies in the development of new therapeutics. However, we are hopeful that targeted protein degradation will be broadly applicable in turning ineffectual ligands into effective degraders able to address all aspects of target protein function.

Targeting Histone Methylation in Human Cancers

Polycomb repressive complexes

The polycomb repressive complexes, PRC1 and PRC2, are transcriptional co-repressors that function via chromatin compaction. Polycomb-mediated gene silencing is initiated by the histone methyltransferase (HMT) subunit of PRC2, EZH2 (enhancer of zeste homolog), which catalyzes methylation of H3K27. PRC1 recognizes H3K27me3 via chromodomain-containing substrate adapters (CBX2, CBX4, CBX6, CBX7, and CBX8) and effects subsequent gene silencing via modifications to histones and DNA. As comprehensively reviewed [92], EZH2 is implicated as an oncogene and non-oncogene dependency in a variety of cancers, owing to overexpression of *EZH2*, gain-of-function mutations to *EZH2*, and loss-of-function mutations to SWI/SNF complex subunits, which ordinarily oppose PRC2 function. This has inspired a number of clinical development programs targeting the EZH2 methyltransferase and recently, a phase 1 study of tazemetostat (Figure 2), an EZH2 HMT inhibitor, reported nearly 40% response rates in both non-Hodgkin lymphomas and SWI/SNF-mutant solid tumors [93]. Interestingly, EZH2 has also been reported to possess non-enzymatic, scaffolding function that is essential for the survival for SWI/SNF-mutant tumors [94]. In this context, genetic or chemical disruption of methyltransferase activity only partially exploits EZH2 dependency [94], presenting an attractive rationale to develop EZH2-selective PROTACs able to fully phenocopy genetic depletion.

More recently, two groups concurrently revealed first-in-class allosteric inhibitors of PRC2 that bind the WD40 domain of the core PRC2 subunit, EED [95,96]. These structurally distinct small molecules, EED226 and A-395 (Figure 2), potently inhibit methyltransferase activity in vivo and excitingly, are able to overcome mutations to EZH2 that confer resistance to EZH2 HMT inhibitors [95,96]. As yet another alternative, several groups have

endeavored to inhibit the downstream effectors of PRC2-installed H3K27me3 by targeting the chromodomains of CBX family proteins in PRC1 that bind to the repressive histone mark [97,98]. These efforts have focused on peptide-based scaffolds, yielding potent tool compounds, including the chemical probe, UNC3866 (Figure 2) [98]. Whether CBX chromodomains – and chromodomains at large – will be tractable targets for small molecules remains an open question.

Methyltransferases and Hematological Malignancies

Outside of PRC2, a number of other histone methyltransferases have also been described as dependencies in cancer, particularly in hematologic malignancies. DOT1L, the methyltransferase responsible for modifying H3K79, is a well-described dependency in acute leukemia bearing fusion oncogenes involving *lysine methyltransferase 2A* (*KMT2A*) [99-101]. Pinometostat, a clinical-stage DOT1L inhibitor [102], is very well tolerated and shows initial signs of efficacy in early-phase trials of KMT2A-fusion acute leukemia [103]. Other efforts to kill KMT2A-fusion leukemia have focused on the interaction between KMT2A and its cofactor, Menin. Small-molecule inhibitors of Menin-KMT2A are effective in several leukemic subtypes [104,105] and are expected to enter clinical trials soon. ASH1-like histone lysine methyltransferase (ASH1L), which methylates H3K36, has been similarly described as a dependency in KMT2A-fusion-positive leukemia [106], and intriguingly, a series of 3-(1*H*-indol-3-yl)benzothioamide-based inhibitors and PROTACs for ASH1L have been reported in the patent literature [107]. Additionally, the H3K36 HMT, NSD2, is known to be frequently translocated or mutated in various hematologic malignancies resulting in hyperactivity and increased sensitivity to loss of NSD2 [108]. Early efforts in assay development and discovery chemistry for the NSD2 methyltransferase have recently been reported – a likely foreshadowing of future therapeutic development [109].

Histone Demethylation and Drug-Tolerant Persistence

Histone demethylase enzymes, which function antagonistically to histone methyltransferases, are also extensively implicated in cancer pathogenesis, notably including the interesting phenomenon of drug-tolerant persistence. This is a form of non-genetic tolerance to anti-cancer drugs that emerges from rare subpopulations of cancer cells [110,111]. Converging lines of evidence implicate chromatin regulatory rewiring via activity of lysine-specific demethylases, including lysine demethylase 5 (KDM5) and KDM6 families, as important in multiple models of drug-tolerant persistence [110,112-114]. Both KDM5 and KDM6 families have proven druggable and recent efforts have extended into highly potent inhibitors with activity *in vivo* [115,116]. In one report, CPI-455, a selective pyrimidinone inhibitor of the KDM5 family demethylases, effectively diminished the emergence of drug-tolerant persister cells following treatment with distinct cytotoxic anti-cancer drugs in diverse cancer lineages *in vitro* [113]. Extension of this phenotype *in vivo* holds promise as a means of widely suppressing drug resistance mechanisms in the clinic.

Concluding Remarks and Future Perspectives

As witnessed by the examples above, the pharmacological science of targeting transcriptional co-regulators has matured immeasurably. Efforts in discovery chemistry are

profiles, even the most selective compounds often target one or more closely related homologs (*e.g.* CBP/p300 bromodomain and HAT inhibitors target both homologs). Unfortunately, off-target activity on close homologs often limits the ability to develop drugs targeting synthetic lethality in cancer as synthetic lethal relationships are often observed between closely related homologs with redundant or semi-redundant function. It is becoming clear that PROTACs may offer assistance with this challenge as well. On multiple occasions, PROTACs have been discovered to possess improved selectivity compared to the parent ligand, sometimes affecting just a single protein [12,33-35,68,126-128].

Although we have highlighted the potential for targeted protein degradation to overcome significant challenges in co-regulator pharmacology (and drug discovery more broadly), this field still faces a number of challenges itself. Notably, the origin of improved selectivity – when it is observed – is often unclear, highlighting the need to discover generalizable principles governing “degradability” [34,35,129]. Moreover, the bulk of disclosed small-molecule PROTAC molecules have been synthesized by conjugation to one of two E3-targeting ligands. As additional E3-targeting ligands are discovered [130-134], the practical scope of degradable targets is likely to be significantly expanded, which is bound to impact the pharmacological development of transcriptional co-regulator.

Like with most, if not all, molecularly targeted cancer therapeutics, it will be essential to identify appropriate combination therapies able to increase efficacy and suppress the emergence of evasive resistance. Mounting evidence has implicated several transcriptional co-regulators as determining responses to anti-tumor immunotherapy, offering a particularly intriguing setting for combining transcriptionally targeted drug candidates. In less than two years, CDK9, SWI/SNF, and the lysine demethylase, KDM1A have all been identified as targets able to potentiate anti-cancer immunotherapy drugs [11,135-137]

The most important experiment, ongoing in many cases, is the clinical evaluation of drugs targeting coregulators in cancer. Setting aside the histone deacetylase (HDAC) inhibitors that have been approved as anticancer agents by the Food and Drug Administration (FDA) (which we have decided not to discuss here as this target class has been well-reviewed in [138]), the targets discussed in this review have reached only as far as clinical trials. Thus, the results of ongoing clinical trials are anxiously awaited to answer questions of efficacy and therapeutic index in patients. Meanwhile, sophisticated use of genetics and pharmacology will continue to grow the list of transcriptional co-regulators that represent tractable targets for therapeutic intervention.

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Glossary

Auxin-inducible degron:

auxin is a phytohormone that binds the E3 ligase substrate adapter, TIR1, enabling association and eventual degradation of AUX/IAA transcriptional repressors. Expression of TIR1 in mammalian cells, coupled with tagging of a protein of interest (POI) with an epitope from AUX/IAA, enables rapid and reversible auxin-inducible degradation of POI-AUX/IAA fusion proteins.

Cancer-specific dependency:

a vulnerability affecting cancer cell survival that is not present in normal cells and tissues.

Cis-regulatory element:

a regulatory site encoded in DNA that recruits *trans*-acting factors to affect the transcriptional activity of a gene in *cis*. The best studied *cis*-regulatory elements are promoters, located proximally to transcription start sites, and enhancers, which are located distal to the promoter region and act in lineage-specific patterns.

dTAG:

a ligand-inducible degradation system for rapid and reversible control of protein levels in mammalian cells. After tagging a POI with FKBP12^{F36V}, treatment with a small molecule, dTAG-13, induces association of the POI-FKBP12^{F36V} to CRL4^{CRBN}, resulting in degradation of the POI.

IMiD:

thalidomide, lenalidomide, pomalidomide, and related phthalimide analogs are termed “immunomodulatory imid drugs” or IMiDs. These molecules bind to Cereblon (CRBN), the substrate receptor for the CRL4^{CRBN} E3 ubiquitin ligase complex.

Ligandability:

the ability of a protein to support high-affinity interactions with small molecules. This term is similar to druggability, but it does not presume a functional effect of ligand-protein binding.

RING domain:

a protein fold often, but not always, possessing intrinsic E3 activity.

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Box 1.**Genetics of cancer-specific dependencies.**

Cancer-specific dependency refers to protein functions and regulatory pathways that are specifically required for the survival of a cancer cell but are less important to the survival and function of normal cells and tissues. Oncogene dependency, equivalently termed oncogene addiction, refers specifically to a continuous requirement by a cancer cell for the function of its oncogene [6]. Meanwhile, non-oncogene dependency is the concept of requiring the function of a protein or pathway that although not an oncogenic driver of the cancer, is still essential for its growth or survival, but comparatively less important in normal cells and tissues [7]. This concept was proposed based on the hypothesis that certain alterations present in a cancer cell may provoke a deficiency in, or additional need for, some function that does not affect physiological cell survival. Several mechanisms for this are now well-understood, including, but not limited to:

1. Loss-of-function mutations that confer vulnerability to loss of a homolog with some redundancy in protein function.
2. Loss-of-function mutations that confer vulnerability to loss of proteins with opposing regulatory function.
3. Gain-of-function mutations that confer vulnerability to loss of effectors that are required for expression of the gain-of-function phenotype.

The concept of synthetic lethality has been borrowed from classical genetics to describe these classes of nononcogene dependencies in which loss of protein function – via genetic or pharmacological perturbation – is only lethal in the presence of a cancer-associated genetic alteration. Other non-oncogene dependencies may not be as easily defined by a single mutation, but by the combined action of many genetic, transcriptional, and/or post-transcriptional alterations that together, confer a cancer cell state expressing a unique vulnerability. An on-going question for translational cancer research is the best way to demonstrate that a dependency is cancer-specific, such that drugs designed to target that dependency exhibit the anticipated therapeutic index. When animal models for the disease are tractable, this can be answered by measuring effects of protein loss on normal and malignant tissues. However, when animal models are not tractable, panels of cell lines across multiple cancer lineages can be used to demonstrate lineage-restricted essentiality as a surrogate measure of cancer-specific essentiality. High-quality chemical probes for a potential target can be particularly valuable toward these ends and may also have direct relevance as starting points for drug development [139].

Box 2.**SWI/SNF complex.**

The SWI/SNF complex is an evolutionarily conserved, multisubunit chromatin remodeler, first identified in yeast genetic screens and named for *mating type switching* (*swi*) and *sucrose non-fermenting* (*snf*) alleles [140-142]. In mammalian cells, SWI/SNF evolved to a ~2 MDa complex and incorporates one of two mutually exclusive ATPase subunits: SWI/SNF-related, matrix associated, actin-dependent regulator of chromatin subfamily A member 2 (SMARCA2, also Brahma or BRM) and SMARCA4 (also Brahma related gene 1 or BRG1). Also termed BAF (BRG1/BRM-associated factor), mammalian SWI/SNF complexes are able to adopt diversified functions via combinatorial assembly of different homologs across multiple gene families [143]. Moreover, multiple distinct complex formations have been characterized to date, termed BAF, PBAF, and ncBAF [65,66,144]. A remarkable 20% of human cancers feature a mutation to a gene encoding a SWI/SNF subunit [145] and many of these mutations present opportunities to exploit synthetic lethality for the development of anti-cancer therapeutics [146].

OUTSTANDING QUESTIONS:

- How will the first generation of drugs targeting co-regulators perform in the clinic?
- What role will combination therapy play in bringing co-regulator pharmacology to the clinic?
- What common, but as-yet undrugged, co-regulator protein folds might be amenable to pharmacological modulation?
- Considering the unique advantages of targeted protein degradation, but also concerns about the size of bifunctional PROTACs, what role will targeted protein degradation play in bringing co-regulator pharmacology to the clinic?
- What are the underlying principles governing “degradability” and can these principles be applied predictively to achieve selectivity between high-affinity targets?

HIGHLIGHTS:

- Transcriptional co-activators and co-repressors (together, co-regulators) are chromatin-associated proteins that mediate signaling from DNA-bound transcription factors to the activation or suppression of gene transcription.
- An increasing number of co-regulators are annotated as cancer-specific oncogene and non-oncogene dependencies in human malignancies.
- Recent disclosures of drug-like small molecules have revealed many of these proteins, and the cancer-promoting activity encoded within them, to be tractable targets for pharmacological modulation.
- Application of these small-molecule tools is opening new views into the function of co-regulators in transcriptional control and identifying underlying mechanisms by which these genes affect cancer-specific cell survival.

Figure 1. Mechanisms of transcriptional co-regulators.

Transcriptional co-regulators mediate transcription factor signaling via multiple molecular mechanisms. Chromatin remodelers alter nucleosome positioning and composition, chromatin writers add post-translational modifications (shown as orange circles) to histones and other proteins, histone erasers remove such post-translational modifications, and chromatin readers assemble regulatory complexes by anchoring onto histones with specific states of modification.

Figure 2, Key Figure. Targeting transcriptional co-regulators in cancer.

(A) Recent examples of pharmacological agents able to disrupt transcriptional co-regulators. Small green circles indicate acetyl-lysine, small red circles indicate H3K27me3, and the small black circle on RNA Pol II represents post-translational phosphorylation.

Figure 3. The emerging pharmacology of small-molecule protein degraders.

(A) Schematic showing the design of PROTACs. PROTACs are composed of a target protein ligand and an E3 ubiquitin ligase-binding ligand, connected by a linker region. PROTAC induce targeted protein degradation by simultaneously engaging a target protein and an E3 ubiquitin ligase complex, which results in poly-ubiquitination of the target protein and ultimately, proteasome-dependent degradation. (B) BET bromodomain inhibitors (blue ovals) inhibit the protein (BRD4) by occupying its druggable site and consequently disables its interaction with acetyl-lysine side chains (green circles) on chromatin. BRD4 PROTACs

can act sub-stoichiometrically by directing multiple cycles of ubiquitination and degradation and remove the entire protein (BRD4) rather than inhibiting it.

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