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Review

The Mechanisms behind the Therapeutic Activity of BET Bromodomain Inhibition

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The bromodomain and extraterminal (BET) protein Brd4 recruits transcriptional regulatory complexes to acetylated chromatin. While Brd4 is considered to be a general transcriptional regulator, pharmacological inhibition of BET proteins shows therapeutic activity in a variety of different pathologies, particularly in models of cancer and inflammation. Such effects have been attributed to a specific set of downstream target genes whose expression is disproportionately sensitive to pharmacological targeting of BET proteins. Emerging evidence links the transcriptional consequences of BET inhibition to the association of Brd4 with enhancer elements, which tend to be involved in lineage-specific gene regulation. Furthermore, Brd4 engages in direct regulatory interactions with several DNA-binding transcription factors to influence their disease-relevant functions. Here we review the current understanding of molecular mechanisms that underlie the promising therapeutic effects of BET bromodomain inhibition.

Introduction

The bromodomain and extraterminal domain (BET) family of adaptor proteins is comprised of Brd2, Brd3, Brd4, and Brdt, which performs diverse roles in regulating transcription by RNA polymerase II (Pol II). Common to all four BET proteins are two conserved N-terminal bromodomains (BD1 and BD2), which are chromatin interaction modules that recognize acetylated lysine residues on histone tails and other nuclear proteins (Figure 1) (Dhalluin et al., 1999). The bromodomain structure consists of four α helices separated by variable loop regions, which together form a hydrophobic cavity that recognizes acetyl-lysine (Dhalluin et al., 1999). The human genome encodes 61 bromodomains present in 46 different proteins, where differences in the amino acid residues around the acetyl-lysine binding site impart ligand specificity (Filippakopoulos et al., 2012). Bromodomains of the BET family are known to have modest affinity for monoacetylated lysine in a range of polypeptide contexts; however, their affinity is significantly greater when multiple acetylation sites exist within a span of one to five amino acids (Dey et al., 2003; Filippakopoulos et al., 2012; Morinière et al., 2009). In certain settings, this increase in affinity has been attributed to bidentate acetyl-lysine recognition that occurs within a single bromodomain pocket (Filippakopoulos and Knapp, 2012; Gamsjaeger et al., 2011; Morinière et al., 2009). Bromodomain-mediated interactions with acetylated chromatin result in the localization of BET proteins to discrete locations along the chromosome, where they recruit other regulatory complexes to influence gene expression (Figure 2) (Dey et al., 2003).

A well-studied member of the BET family is Brd4, whose role in transcriptional regulation was first suggested by the constituents of its associated protein complex, which included P-TEFb and Mediator (discussed separately below) (Figure 2) (Jang et al., 2005; Jiang et al., 1998; Yang et al., 2005). P-TEFb is a heterodimer of cyclin-dependent kinase 9 (Cdk9) and one of its regulatory subunits, Cyclin T1, T2, or K, which promotes Pol II elongation through its kinase activity (reviewed in Zhou et al., 2012). At a significant fraction of metazoan genes, recently initiated Pol II becomes arrested, or paused, within ~60 nucleotides of the transcription start site through a variety of different means (Kwak and Lis, 2013). One such mechanism is through interactions with regulatory factors DSIF and NELF, which can maintain Pol II in a reversibly paused state (Adelman and Lis, 2012). When recruited to the promoter-proximal region, P-TEFb can phosphorylate DSIF and NELF to inhibit their function and allow productive transcription elongation (Zhou et al., 2012). P-TEFb also phosphorylates the heptad repeats of Pol II at the serine-2 position, a modification also associated with active elongation (Zhou et al., 2012).

At least two regions of Brd4 bind directly to P-TEFb: the C-terminal domain (CTD) interacts with Cyclin T1 and Cdk9, while BD2 recognizes an acetylated region of Cyclin T1 (Bisgrove et al., 2007; Jang et al., 2005; Schröder et al., 2012). The interaction with Brd4 will prevent P-TEFb from associating with 7SK/HEXIM, a ribonucleoprotein complex that sequesters P-TEFb in a kinase-inactive state (Jang et al., 2005; Yang et al., 2005). Thus, the association with Brd4 can modulate the global level of P-TEFb activity in the cell (Jang et al., 2005; Yang et al., 2005). Brd4 also has an intrinsic kinase activity that can phosphorylate the heptad repeats of Pol II at serine-2 in vitro, suggesting additional mechanisms through which Brd4 might regulate Pol II elongation (Devaiah et al., 2012). However, the biochemical mechanism underlying this kinase activity is unclear at present, since Brd4 lacks homology to other known kinase domains. Nonetheless, these findings have implicated Brd4 as a general regulator that couples the acetylation state of chromatin with Pol II elongation (Jang et al., 2005; Yang et al., 2005). The Brd4/P-TEFb interaction is particularly important for rapid transcriptional induction, as occurs at mitotic exit and in response to signal-dependent activation of DNA-binding

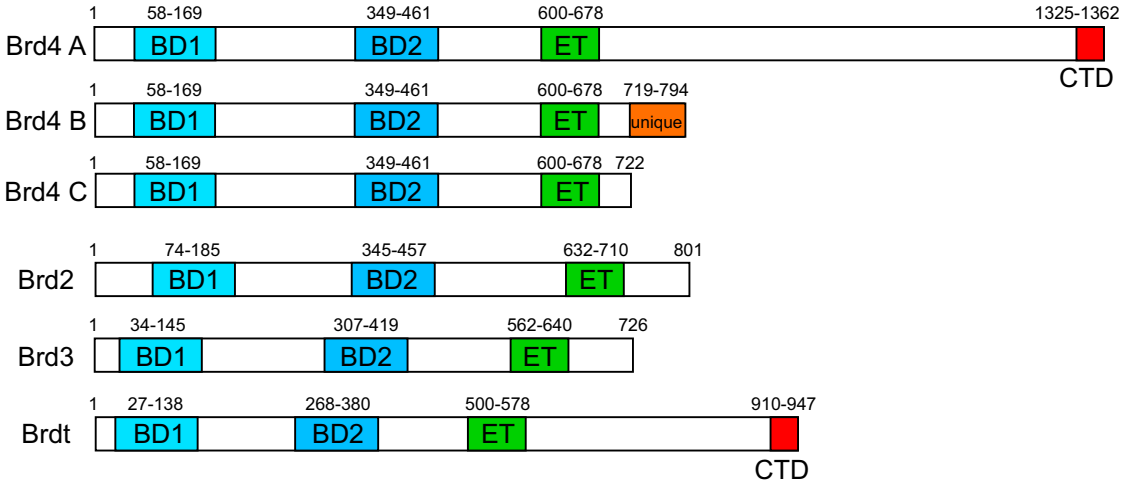

Figure 1. Domain Architecture of Human BET Proteins

Three isoforms of Brd4 are indicated. ET, extraterminal domain. CTD, C-terminal domain. Isoform B of Brd4 has a unique C terminus, which interacts with condensin II complexes (Floyd et al., 2013). Brdt and Brd2 have multiple isoforms, which are not indicated here. In some studies, the CTD of Brd4 and Brdt is referred to as a C-terminal motif (CTM).

transcription factors (TFs) (Dey et al., 2009; Hargreaves et al., 2009; Yang et al., 2008; Zippo et al., 2009).

Brd4 was identified in one of the original purifications of the mammalian Mediator complex, a multiprotein coactivator that links TFs to Pol II activation (Jiang et al., 1998). Consistent with this interaction, Brd4 and Mediator occupy similar sites across the genome and can stabilize one another's occupancy at certain regions (Donner et al., 2010; Lovén et al., 2013). While the Brd4/Mediator association has been widely observed, the binding surfaces that link these two factors have yet to be defined. Nonetheless, the interaction with Mediator suggests that Brd4 could provide an interface between TFs and the transcriptional apparatus (Wu and Chiang, 2007).

The ET domain of Brd4 has been linked to transcriptional regulation by interacting with several histone modifiers, including the arginine demethylase Jmjd6 and the lysine methyltransferase Nsd3 (Liu et al., 2013; Rahman et al., 2011). In addition, the ET domain has been found to associate with ATP-dependent nucleosome-remodeling enzymes SWI/SNF and CHD4 (Rahman et al., 2011). These interactions imply that Brd4 alters chromatin structure at its occupied sites, although the regulatory significance of these associations is currently not well understood.

Therapeutic Targeting of BET Bromodomains with Small Molecules

In 2010, two independent groups reported small-molecule inhibitors of BET bromodomains, called JQ1 and I-BET, which have a similar chemical structure and mode of target inhibition (Filippakopoulos et al., 2010; Nicodeme et al., 2010). JQ1 and I-BET are notable for their high affinity for bromodomains of the BET family (BD1 and BD2 of Brd2/Brd3/Brd4/Brdt) over other bromodomain subfamilies. Both BET inhibitors engage the bromodomain pocket in a manner that is competitive with acetylated peptide binding, thereby causing the displacement of all four BET proteins from chromatin in cells upon exposure to these compounds (reviewed in Prinjha et al., 2012). BET inhibitors also have suitable pharmacokinetics for in vivo application, which has enabled a rapid evaluation of their therapeutic activity in various disease models.

BRD4 can be mutated by chromosomal translocation to form in-frame fusions with the *NUT* gene to initiate an aggressive cancer called midline carcinoma (French, 2012). The resulting Brd4-NUT oncoprotein is an aberrant transcriptional regulator that relies on the bromodomains of Brd4 for its oncogenic function. Hence, this malignancy provided a clear rationale for the initial therapeutic evaluation of BET inhibitors. In midline carcinoma cell lines, JQ1 treatment led to the release of Brd4-NUT from chromatin and triggered terminal squamous cell differentiation and apoptosis (Filippakopoulos et al., 2010). In patient-derived xenograft models of midline carcinoma, it was shown that daily exposure to JQ1 extended the survival of cancer-bearing mice at doses that had minimal toxicity to normal tissues (Filippakopoulos et al., 2010). This pivotal study provided the first demonstration of efficacy for a BET inhibitor in a preclinical cancer model.

Since midline carcinoma is a rare form of cancer, a major effort has also been directed at targeting BET proteins in other malignancies that lack *BRD4* rearrangments. Studies from several laboratories, including our own, have identified therapeutic effects for BET inhibitors in mouse models of hematological malignancies, such as acute myeloid leukemia, multiple myeloma, and lymphoma (Dawson et al., 2011; Delmore et al., 2011; Mertz et al., 2011; Zuber et al., 2011). Unlike in midline carcinoma, the therapeutic activity of BET inhibitors in hematological cancers can span diverse genetic subtypes of disease and generally is attributed to targeting the protein product of a wild-type *BRD4* gene. Exposure of malignant blood cells to BET inhibitors leads to the rapid transcriptional suppression of key proto-oncogenes, such as *MYC* and *BCL2* (Dawson et al., 2011; Delmore et al.,

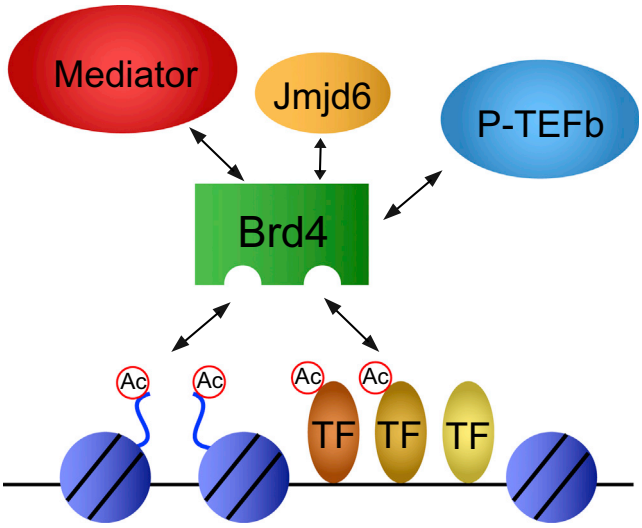

Figure 2. Protein-Protein Interactions Involving Brd4
Depicted are some of the critical protein-protein interactions employed by Brd4 that could be relevant to the therapeutic effects of BET inhibitors. Mediator, Jmjd6, and P-TEFb are candidate effectors recruited by Brd4 to regulate transcription by Pol II. Acetylated histones and/or TFs have been suggested as potential recruiters of Brd4 to promoters and/or enhancer regions.

2011; Mertz et al., 2011; Zuber et al., 2011). Importantly, overexpression of either *MYC* or *BCL2* from a heterologous promoter can partially alleviate the anticancer effects of JQ1/I-BET, highlighting that suppression of these genes is an important downstream effect of BET inhibition in these malignancies (Dawson et al., 2011; Delmore et al., 2011; Mertz et al., 2011; Zuber et al., 2011).

In addition to hematological cancers, emerging evidence suggests that certain solid tumors are also sensitive to BET inhibitors. For example, proliferation of a subset of non-small-cell lung cancer cell lines is inhibited by JQ1, effects correlating with suppression of *FOSL1* expression (Lockwood et al., 2012). Neuroblastomas that harbor *NMYC* amplifications are also sensitive to JQ1, correlating with suppression of *NMYC* transcription (Puissant et al., 2013). In Table S1, available online, we have listed the preclinical studies reported to date that show therapeutic activity of BET inhibitors in various diseases. A picture emerges across these studies where the anticancer activity of BET inhibitors can sometimes be correlated with effects on *MYC* transcription, but in other instances these effects are mediated by a different set of cancer-relevant genes. It should be noted that in the majority of these studies the therapeutic effect of BET inhibitors has been attributed to targeting Brd4, rather than the other BET proteins. Based on the promising effects seen in these preclinical studies, several phase I clinical trials commenced in 2013 to evaluate the safety and efficacy of BET inhibitors in human cancer patients (ClinicalTrials.gov Identifiers NCT01587703, NCT01713582, NCT01949883, NCT01987362, and NCT01943851).

BET inhibitors also exhibit potent anti-inflammatory effects in vivo, with a single injection of I-BET preventing lethal septic shock induced by lipopolysaccharide (LPS) (Nicodeme et al., 2010). This therapeutic effect has been linked to an essential role of Brd2, Brd3, and Brd4 in the induction of inflammatory gene transcription (Belkina et al., 2013; Hargreaves et al., 2009; Nicodeme et al., 2010). Treating macrophage cells with I-BET prevented the activation of a specific subset of LPS-inducible genes that encode cytokines, chemokines, and various TFs involved in the inflammatory response (Nicodeme et al., 2010). In the absence of LPS stimulation, I-BET treatment will lead to minimal changes to global gene expression in macrophages, indicating selective effects of BET inhibitors on inflammatory genes in this cell type (Nicodeme et al., 2010). BET inhibitors have also been shown to influence the differentiation of T helper cells following self-antigen presentation, which can lead to therapeutic effects in mouse models of autoimmune disease (Bandukwala et al., 2012; Mele et al., 2013).

In addition to effects on cancer and immune processes, other organ systems are also influenced by pharmacological BET inhibition. In the cardiovascular system, JQ1 treatment has been shown to prevent heart failure by altering a Brd4-dependent transcriptional program linked to cardiomyocyte hypertrophy (Anand et al., 2013; Spiltoir et al., 2013). BET inhibitors also inhibit spermatogenesis, which may occur through targeting of the testes-specific BET protein Brdt (Matzuk et al., 2012). Collectively, these studies reveal how targeting BET proteins can have diverse effects on mammalian physiology and pathophysiology, representing several opportunities for further clinical evaluation.

Brd4 has been described as a general regulator for Pol II-dependent transcription through interactions with P-TEFb (Jang et al., 2005; Yang et al., 2005). Consistent with this possibility, JQ1 exposure causes a modest reduction in global mRNA levels and in total Pol II serine-2 phosphorylation (Anand et al., 2013; Devaiah et al., 2012; Lovén et al., 2013). However, the studies with BET inhibitors described above indicate that not all genes are equally affected by JQ1, but that instead transcription is disproportionately suppressed at specific sites. It should be emphasized that the transcriptional effects of BET inhibitors are often highly specific to the cell type being examined. For example, treating leukemia cells with JQ1 causes dramatic *MYC* suppression, whereas treating fibroblast cells with JQ1 has a minimal impact on *MYC* levels (Zuber et al., 2011). The molecular mechanisms of BET protein function that underlie these therapeutic effects have been the subject of several recent studies, described below.

Role of Brd4 in Enhancer-Mediated Gene Regulation

Insight into the disease-relevant functions of Brd4 has been obtained from genome-wide analysis of its chromatin occupancy, using chromatin immunoprecipitation coupled with massively parallel DNA sequencing (ChIP-seq). Consistent with its presumed role as a general regulator, Brd4 has been found to associate with essentially all active promoters and a significant fraction of active enhancers in the genome of various normal and transformed cell types (Anand et al., 2013; Lovén et al., 2013; Zhang et al., 2012). The overall pattern of Brd4 occupancy correlates with that of several different histone acetylation marks (e.g., H4K5, H4K8, H3K9, and H3K27), consistent with a bromodomain-mediated recruitment mechanism (Lovén et al., 2013; Zhang et al., 2012).

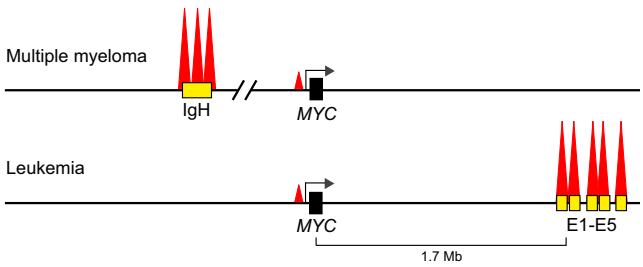

Figure 3. Lineage-Specific Arrangements of Brd4-Occupied Enhancers at the *MYC* Locus that Correlate with Sensitivity to Pharmacological BET Inhibition

Brd4 occupancy is represented by red triangles as measured by ChIP-seq in either MM1.S cells (top) (Lovén et al., 2013) or in MLL-AF9/Nras^{G12D} acute myeloid leukemia (Shi et al., 2013). In these two cell lines, *MYC* transcription is highly sensitive to BET inhibition.

Examination of Brd4 occupancy at genes whose transcription is particularly sensitive to JQ1 has led to the observation that such genes often exhibit high levels of Brd4 occupancy at nearby enhancer regions. This was first noted at the *MYC* locus in the multiple myeloma cell line MM1.S, where a translocation with the IgH enhancer region drives high *MYC* expression to promote disease progression. In this particular cell line, *MYC* transcription has been shown to be exquisitely sensitive to JQ1-mediated suppression (Delmore et al., 2011). Notably, the level of Brd4 occupancy at the IgH enhancers is more than 10-fold greater than that seen at the *MYC* promoter, suggesting that enhancers might be the major source of Brd4-dependent transcriptional activation (Figure 3) (Delmore et al., 2011). In leukemia cells, *MYC* transcription is also highly sensitive to JQ1 in the absence of genomic rearrangements of the *MYC* locus (Dawson et al., 2011; Mertz et al., 2011; Zuber et al., 2011). Leukemia cells possess a unique set of Brd4-occupied enhancers, which are located 1.7 Mb downstream of the *MYC* promoter (Figure 3) (Shi et al., 2013). These lineage-specific enhancers are occupied by several hematopoietic TFs and display long-range looping interactions with the *MYC* promoter (Shi et al., 2013). While *MYC* is expressed ubiquitously in most proliferating cell types, the enhancers that regulate this gene are highly cell type specific, which raises the possibility that different enhancer configurations at the *MYC* locus might be differentially sensitive to BET bromodomain inhibition (Figure 3) (Hnisz et al., 2013; Kieffer-Kwon et al., 2013; Shi et al., 2013).

While Brd4 occupies thousands of putative enhancer elements in a given cell type, it has been noted that only a small fraction of enhancers in MM1.S cells (~300) exhibit the high level of Brd4 occupancy seen at the IgH region (Lovén et al., 2013). These elements have been termed "super-enhancers" and are remarkable for the level and the span of Brd4 occupancy, which can extend across tens of kilobases (Lovén et al., 2013; Whyte et al., 2013). The unusual size of super-enhancers is reminiscent of locus control regions, e.g., at β-globin locus, which are clusters of regulatory elements known to drive high levels of lineage-specific transcription (Li et al., 1999). Several studies describe a similar phenomenon in which a specific subset of regulatory DNA elements exhibits high levels of occupancy for TFs, histone modifications, and/or coregulators (e.g., stretch enhancers and HOT regions) (modENCODE Consortium et al., 2010; Parker et al., 2013; Yan et al., 2013). However, it remains an open question whether enhancers that are loaded with exceptional levels of regulatory factors share any specialized functional properties.

It has been proposed that genes regulated by super-enhancers are particularly sensitive to BET inhibition (Lovén et al., 2013). This is based on the observation that the mRNA level of genes located near super-enhancers tend to be more downregulated by JQ1 than genes located near typical enhancers, based on genomewide analyses performed in MM1.S cells and subsequently in Ly1 lymphoma cells (Chapuy et al., 2013; Lovén et al., 2013). While the magnitude of this differential effect was quite small, a lack of consideration for differences in mRNA half-life and the assumption that enhancers regulate the nearest expressed gene may have obscured the strength of this association. Nonetheless, independent reports of Brd4-dependent genes having nearby super-enhancers lend additional support that the transcriptional effects of BET inhibitors can be linked to large domains of Brd4 occupancy, at least for certain genes (Dawson et al., 2014; Gröschel et al., 2014; Shi et al., 2013). An important goal for future investigation will be to clarify the generality and the robustness of the relationship between super-enhancers and the transcriptional effects of BET inhibition.

While the role of Brd4 in promoting Pol II elongation at promoter-proximal regions is well established, the regulatory function of Brd4 at enhancer regions is less clear. A recent study suggests that Brd4 might form distinct complexes when bound to promoters versus enhancers, with the latter containing the demethylase protein Jmjd6 (Liu et al., 2013). Brd4 was found to recruit Jmjd6 to enhancers where it demethylates two distinct substrates: the 5′ methyl-phosphate cap of 7SK RNA and histone H4 arginine 3 mono- and dimethylation (H4R3me1 and H4R3me2), a chromatin mark associated with transcriptional repression (Zhao et al., 2009). Since 7SK relies on its methyl-phosphate cap to evade exonucleolytic degradation (Jeronimo et al., 2007), recruitment of Jmjd6 would cause degradation of 7SK as an additional means by which Brd4 could promote the local activation of P-TEFb (Liu et al., 2013). Long-range chromatin-looping interactions would then allow enhancer-bound Brd4/Jmjd6/P-TEFb complexes to promote pause release of Pol II at the target promoter-proximal region (Liu et al., 2013). The authors also showed that knockdown of Brd4 and Jmjd6 led to similar global gene expression changes, consistent with both factors operating in a complex with one another (Liu et al., 2013). One provocative observation in the study by Liu et al. (2013) was that 7SK RNA was found to interact with histone tails marked by H4R3me2. Such an interaction, if independently verified, would provide the first example of an RNA functioning as a chromatin reader module, with important implications as a novel element of chromatin regulation.

Direct Brd4 Interactions with Transcription Factors

An enhancer DNA element is essentially comprised of clustered TF binding sites, which form a platform for the recruitment of transcriptional regulatory complexes (Calo and Wysocka, 2013). The presence of Brd4 at enhancers would suggest that its function could be linked to the TFs associated with these

sites. As a general rule, TFs recruit acetyltransferases (e.g., Cbp and Gcn5) as coactivators, which results in lysine acetylation of enhancer-associated nucleosomes and other nonhistone proteins, including the TFs themselves. In one scenario, Brd4 recruitment to enhancers or promoters might be largely determined by the combinatorial pattern of histone acetylation, which would be established indirectly by TF-mediated acetyltransferase recruitment. Alternatively, Brd4 recruitment to chromatin could be determined by direct interactions with the TFs themselves. While these two models are not mutually exclusive, recent studies described below show that Brd4 engages in direct contacts with certain TFs, which can occur in either a bromodomain-dependent or -independent manner. Regulatory Brd4/TF interactions might also contribute to the therapeutic transcriptional effects of pharmacological BET inhibition observed in certain disease states.

A biochemical screen was recently performed that evaluated whether Brd4 directly interacts with various purified factors, including preinitiation complex components, chromatin regulators, and sequence-specific TFs (Wu et al., 2013). In addition to confirming the known interaction with P-TEFb, this analysis revealed that Brd4 binds directly to a specific subset of TFs. These interactions presumably occurred in an acetylation-independent manner, since the TFs were expressed and purified from *E. coli*. This group of Brd4-interacting TFs included p53, YY1, c-Jun, AP2, C/EBPα, C/EBPβ, and the Myc/Max heterodimer (Wu et al., 2013). In these experiments Brd4 failed to associate with the majority of TFs tested, including the strong activator Gal4-VP16, suggesting that Brd4 has an intrinsic binding specificity for certain TFs.

In follow-up to this biochemical screen, the authors demonstrated that the C-terminal regulatory domain of p53 interacts with two distinct regions of Brd4 outside of the bromodomains, with one of these interactions requiring pre-existing phosphorylation of Brd4 by casein kinase II (CK2) (Wu et al., 2013). In the absence of phosphorylation, Brd4 engages in a nonproductive interaction with p53 in a complex that cannot associate with DNA. Upon phosphorylation by CK2, the second binding surface of Brd4 became available to interact with p53, which formed a complex that can now associate with DNA and facilitate transcriptional activation (Wu et al., 2013). The phosphorylation of Brd4 also alleviated autoinhibition of its second bromodomain, thereby allowing its interaction with acetylated chromatin. This important study demonstrated a gene-specific functionality for Brd4 via bromodomain-independent TF interactions and demonstrated how such interactions can be regulated by signal transduction cascades (Wu et al., 2013).

In addition to recognizing acetylated histones, the bromodomains of Brd4 can also interact with specific acetylated regions of TFs. One example of a TF that utilizes Brd4 as a coactivator is TWIST, a basic helix-loop-helix TF that controls mesoderm formation during normal development and also epithelial-to-mesenchymal transitions (EMT) during cancer progression (Shi et al., 2014). TWIST can be diacetylated by the TIP60 acetyltransferase at a motif ($GK^{ac}RGK^{ac}$) which resembles the diacetylation site on histone H4 ($GK^{ac}GGK^{ac}$). When acetylated, TWIST was found to associate specifically with BD2 of Brd4 and not other bromodomains (Shi et al., 2014). Using NMR spectroscopy, critical residues in the bromodomain pocket were identified that could explain the specificity of the TWIST/BD2 interaction. In functional assays, lysine-to-arginine substitutions of these two acetylation sites led to defects in TWIST-dependent induction of EMT (Shi et al., 2014). The authors proposed a model where Brd4 simultaneously associates with acetylated histones (via BD1) and acetylated TWIST (via BD2) at enhancer regions to facilitate P-TEFb-mediated transcriptional activation. The interaction with TWIST implicates a role for Brd4 in the biology of epithelial cancers, which is possibly related to the therapeutic effects of BET inhibitors observed in xenograft models of breast cancer (Shi et al., 2014).

The hematopoietic TF GATA-1 is another example in which acetylation of a histone-like sequence motif can facilitate interactions with BET proteins. GATA-1 is diacetylated by CBP at a motif ($GK^{ac}GKK^{ac}$) that also resembles histone H4, which creates a specific docking site for BD1 of Brd3 (Gamsjaeger et al., 2011; Lamonica et al., 2011). Point mutations that remove these acetylation sites prevented GATA-1 from occupying its cognate DNA elements in a chromatinized template, but not on naked DNA (Lamonica et al., 2006). Furthermore, treating erythroid cell lines with a BET inhibitor resulted in GATA-1 displacement from chromatin and prevented the induction of erythroid-specific gene expression (Lamonica et al., 2011). These studies highlight how BET proteins can be required to stabilize TF occupancy on chromatin, in addition to promoting downstream effects on Pol II activation.

NFκB is a heterodimeric TF that plays an important role in the immune system and in the pathogenesis of cancer (Hayden and Ghosh, 2012). Ligand binding to cell-surface receptors (e.g., LPS binding to TLR4 or TNFα binding to TNFR1) activates signaling cascades that trigger entry of NFκB into the nucleus and the rapid activation of inflammation-associated gene transcription (Hayden and Ghosh, 2012). Original studies with I-BET showed that inhibiting BET proteins in macrophage cells will attenuate the induction of inflammation-associated genes following LPS exposure, suggesting effects on the NFκB pathway (Nicodeme et al., 2010). Indeed, one of the subunits of NFκB, RelA, can be acetylated by the p300 acetyltransferase at lysine 310 to facilitate interactions with both bromodomains of Brd4 (Huang et al., 2009). While the affinity of NFκB K310 acetylation for Brd4 is evidently quite weak, this lysine residue is essential for both Brd4 and P-TEFb recruitment to NFκB target genes following to TNF-α stimulation (Huang et al., 2009). Treating cells with JQ1 will disrupt the RelA-Brd4 interaction and lead to proteasome-mediated RelA degradation, which may further suppress the NFκB pathway (Zou et al., 2013). Gene expression analysis of JQ1-treated cardiomyocytes identified systematic downregulation of NFκB target genes, further suggesting that BET inhibitors can attenuate NFκB function (Anand et al., 2013). These observations raise the possibility that the anti-inflammatory and the anticancer effects of BET inhibitors are mediated through suppression of the NFκB pathway.

It should be noted that NFκB activation with LPS is also accompanied by robust GCN5-mediated H4K5/K8 hyperacetylation at inflammation-associated gene promoters, which occurs coincident with Brd4 recruitment (Hargreaves et al., 2009). Based on this, one cannot easily discriminate whether it is histone or

RelA acetylation that drives inducible Brd4 recruitment to these locations upon LPS stimulation. In fact, JQ1 treatment prevents several different inducible transcriptional responses, including those caused by interferon-γ-mediated activation of STAT TFs and the rapid gene reactivation that occurs at the mitosis-G1 transition (Patel et al., 2013; Zhao et al., 2011). Moreover, the specific subset of inflammatory genes that are suppressed by BET inhibitors tend to exhibit a similar pattern of histone modifications and a low CpG content at their associated promoter region (Nicodeme et al., 2010). Taken together, these observations suggest that the sensitivity of inflammatory genes to BET inhibition is due to multiple factors: the disruption of specific Brd4-TF complexes and the unique chromatin ground state found at the promoters of inducible genes.

Role of Brd4 in Regulating Viral Gene Expression

Latency of HIV in a transcriptionally silent state is a major mechanism through which this virus can evade antiretroviral therapies. Hence, pharmacological strategies aimed at reactivating HIV transcription represent a means of eliminating persistent viral reservoirs. Brd4 has been shown to regulate multiple steps of HIV transcription (Jang et al., 2005; Yang et al., 2005), and a series of recent studies have shown that BET inhibitors can trigger HIV transcription in latently infected cells, thus activating viral replication (Banerjee et al., 2012; Boehm et al., 2013; Li et al., 2013; Zhu et al., 2012). This seemingly paradoxical effect can be explained by the known competition between Brd4 and the viral transactivator, Tat, for their association with P-TEFb (Yang et al., 2005). By inhibiting Brd4 with JQ1, a larger pool of P-TEFb now becomes available to associate with Tat to activate transcription elongation of the HIV genome (Li et al., 2013; Zhu et al., 2012). The mechanism of JQ1-induced HIV transcription might additionally involve inhibition of Brd2, which could act as an endogenous negative regulator HIV latency (Boehm et al., 2013). These studies highlight how BET inhibitors can indirectly lead to transcriptional effects by altering the available pool of P-TEFb in the cell, which also may occur at host genes as well (Bartholomeeusen et al., 2012).

Virally encoded DNA binding proteins also associate with Brd4 to control various aspects of the viral life cycle (reviewed in Weidner-Glunde et al., 2010). For example, HPV encodes a DNA-binding protein called E2, which directly interacts with the CTD of Brd4 to regulate expression of viral genes (Wu et al., 2006; You et al., 2004). In this specific context Brd4 functions as a corepressor that can block preinitiation complex assembly in an acetylation-dependent manner (Wu et al., 2006). The E2-Brd4 interaction can also be exploited by HPV to tether viral episomes to segregating mitotic chromosomes to ensure virus persistence during cell division (You et al., 2004). The use of Brd4 as a host cell factor for numerous viruses justifies further investigation of the safety and efficacy of BET inhibitors in models of acute and chronic infection.

Perspective and Future Directions

BET proteins have emerged in recent years as therapeutic targets in a remarkable range of disease models, which may relate to Brd4 being an integral component of several disease-associated gene regulatory networks. However, the effects of BET inhibitors on numerous transcriptional pathways raise obvious concerns about the tolerability of BET inhibitors in humans, issues that will ultimately become clarified as clinical trials proceed. Nevertheless, one important goal for future studies will be to determine whether more selective perturbations of BET protein function will have improved therapeutic efficacy. Discrimination between BD1 and BD2 pharmacologically presents one opportunity to achieve more selective transcriptional effects. A recent study identified BET inhibitors with a preference for BD2 over BD1, and such compounds led to minimal transcriptional effects when compared to JQ1 (Picaud et al., 2013). Selective inhibitors of individual BET family members would also be anticipated to cause distinct effects on transcription, since the genome occupancy patterns Brd2/Brd3/Brd4 are not identical (Anders et al., 2014). Brd2 has been linked to metabolic regulation and lymphomagenesis; however, little is known about the biological function of Brd3 in vivo (Greenwald et al., 2004; Wang et al., 2010). Since the bromodomains of BET proteins share ~75% identity between family members, it remains uncertain whether highly selective chemical probes will be achievable. Small-molecule targeting of Brd4-interacting proteins might be an additional strategy to perturb transcription therapeutically in a manner with improved selectivity. The feasibility of targeting jumonji domains has recently been demonstrated (Kruidenier et al., 2012); hence Jmjd6 now emerges as a potential drug target in diseases where Brd4 has an established function. In this regard, a comprehensive investigation of factors that work upstream of Brd4 (e.g., acetyltransferases and TFs) or downstream (e.g., ET-associated factors) in different disease contexts may reveal additional opportunities for therapeutic intervention.

Much of our current mechanistic understanding of BET proteins as drug targets is derived from experiments that correlate pharmacological inhibition with global gene expression changes and/or patterns of Brd4 occupancy on chromatin. A clear objective for future investigation will be to decisively demonstrate the critical protein-protein interactions, downstream target genes, and genomic binding sites that are relevant to the role of BET proteins in disease processes. In this regard, defining the mechanisms of resistance to BET inhibitors in cancer models would be a powerful means of revealing the most critical disease-relevant functions of BET proteins. Continued efforts to understand the role of Brd4 in disease states might also inform how we assess other candidate chromatin regulator drug targets, particularly since Brd4 exemplifies how seemingly general regulators can elicit specific biological effects when targeted pharmacologically.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one table and can be found with this article at http://dx.doi.org/10.1016/j.molcel.2014.05.016.

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Table S1. List of Therapeutic Studies Evaluating BET Inhibitors

BET inhibitor	Relevant BET protein target	Disease type	Disease subtype	Mouse model	PMID
JQ1	Brd4-NUT	cancer	midline carcinoma	patient sample xenograft	20871596
JQ1	Brd4	cancer	acute myeloid leukemia	genetically engineered mouse (GEM) model with MLL-AF9 oncogene	21814200
JQ1	Brd4	cancer	Burkitt's lymphoma and acute myeloid leukemia	human cell line xenograft	21949397
I-BET151	Not demonstrated	cancer	acute myeloid leukemia	human cell line xenograft	21964340
JQ1	Brd4	cancer	Multiple myeloma	human cell line xenograft	21889194
JQ1	Brd2/3/4	cancer	glioblastoma	patient sample xenograft	23403638
JQ1	Not demonstrated	cancer	prostate	Pten deficient and p53 null GEM model	24444712
JQ1	Brd4	cancer	lung cancer	human cell line xenograft	23686307
JQ1	Not demonstrated	cancer	B-cell acute lymphoblastic leukemia	patient sample xenograft	22904298
JQ1	Brd4	cancer	Neuroblastoma	human cell line/primary sample xenograft and Mycn-amplified GEM model	23430699
MS417	Brd4	cancer	melanoma	human cell line xenograft	23950209
JQ1	Brd4	cancer	Diffuse Large B Cell Lymphoma	human cell line xenograft	24332044
JQ1/MS417	Brd4	cancer	breast cancer	human cell line xenograft	24525235
JQ1	Not demonstrated	cancer	T-cell acute lymphoblastic leukemia	Tal1/Lmo2 GEM model	24394663
JQ1	Brd4	cancer	Malignant Peripheral Nerve Sheath Tumor	Nf1 null and p53 null GEM model	24373973
JQ1	Brd4	cancer	Medulloblastoma	primary sample xenograft	24297863
JQ1	Brd4	cancer	Medulloblastoma	human cell line xenograft	24231268
JQ1	Brd4	cancer	acute myeloid leukemia	IDH2 R172K GEM model	24065765
JQ1	Brd4	cancer	effusion lymphoma	human cell line xenograft	23792448
I-BET 762	Not demonstrated	cancer	prostate	human cell line xenograft	24293458
I-BET 151	Brd4	cancer	acute myeloid leukemia	NPM1c GEM model	24220271
I-BET 762	Brd4	cancer	Neuroblastoma	human cell line xenograft	24009722
JQ1	Not demonstrated	cancer	B-cell acute lymphoblastic leukemia	human cell line xenograft	23872705
I-BET 151	Brd4	cancer	glioblastoma	human cell line xenograft	24496381
JQ1	Brd4	cancer	acute myeloid leukemia	human cell line xenograft	24435446
JQ1	Brd4	cancer	T-cell leukemia	Rat-1-Tax GEM model	24189064
I-BET151/I-BET762	brd4	cancer	myeloma	human cell line xenograft	24335499
I-BET 151	Brd4	cancer	Breast cancer	murine cell line xenograft	24260471
JQ1 and I-BET762	Brd4	cancer	prostate cancer	human cell line xenograft	24759320
JQ1	Brd4	cardiovascular	Heart Failure	TAC/PE infusion mimic condition of heart failure	23911322
JQ1	Brd4	cardiovascular	Heart Failure	TAC mimic condition of heart failure	23939492
JQ1	Brd2/4	fibrosis	lung fibrosis	Bleomycin-induced lung fibrosis	23115324
JQ1	Brd4	fibrosis	lung fibrosis	Bleomycin-induced lung fibrosis	23759512
I-BET	Brd2/3/4	inflammation/immune	endotoxic shock and sepsis	LPS-, heat-killed bacteria- or caecal ligation puncture stimulations	21068722
JQ1	Brd2/3/4	inflammation/immune	endotoxic shock	LPS stimulation	23420887
MS417	Brd4	inflammation/immune	chronic kidney inflammation	HIV -1 transgenic mice (Tg26)	22645123
I-BET762	Not demonstrated	inflammation/immune	autoimmunity	adoptive transfer T cells in EAE disease model	22912406
JQ1	Brd2/4	inflammation/immune	autoimmunity	CIA/EAE disease models of T cell autoimmune	24101376