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Biological function and histone recognition of family IV bromodomain-containing proteins

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Abstract

Bromodomain proteins function as epigenetic readers that recognize acetylated histone tails to facilitate the transcription of target genes. There are approximately 60 known human bromodomains, which are divided into 8 sub-families based on structural conservation. The bromodomain-containing proteins in family IV include 7 members (BRPF1, BRPF2, BRPF3, BRD7, BRD9, ATAD2 and ATAD2b). The bromodomains of each of these proteins recognize and bind acetyllysine residues on histone tails protruding from the nucleosome. However, the histone marks recognized by each bromodomain protein can be very different. The BRPF1 subunit of the MOZ histone acetyltransferase (HAT) recognizes acetylated histones H2AK5ac, H4K12ac, H3K14ac, H4K8ac and H4K5ac. While the bromodomain of BRD7, a member of the SWI/SNF complex, was shown to preferentially recognize acetylated histones H3K9ac, H3K14ac, H4K8ac, H4K12ac and H4K16ac. The bromodomains of BRPF2 and BRPF3 have similar sequences, and function as part of the HBO1 HAT complex, but there is limited data on which histone ligands they bind. Similarly, there is little known about the histone targets of the BRD9 and ATAD2b bromodomain proteins. Interestingly, the ATAD2 bromodomain was recently shown to preferentially bind to the di-acetylated H4K5acK12ac mark found in newly synthesized histones following DNA replication. However, despite the physiological importance of the family IV bromodomains, little is known about how they function at the molecular or atomic level. In this review we summarize our understanding of how family IV bromodomains recognize and select for acetyllysine marks and discuss the importance of acetylated histone recognition for their biological functions.

Keywords

Acetyllysine; bromodomain; chromatin reader domain; epigenetics; histone

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Introduction

Epigenetics is described as the study of changes in gene expression without altering the DNA sequence (Wolffe and Matzke, 1999). There are three mechanisms known to initiate and sustain epigenetic changes: DNA methylation, RNA-mediated gene silencing and histone modifications (Wolffe and Matzke, 1999). These epigenetic systems are in place to regulate gene expression, however, when gene expression becomes altered it can lead to disease, especially cancer (Wolffe and Matzke, 1999). Three meters of DNA is packaged into each cell through the formation of higher ordered chromatin structures, which are created by first wrapping the DNA around nucleosomes, followed by further compaction of these structures into arrays (Thomas, 1984). Chromatin comes in two varieties, euchromatin and heterochromatin. Euchromatin is open and coincides with active transcription, while the heterochromatin is closed and is transcriptionally inactive (Hennig, 1999). Nucleosomes are octomeric complexes that contain a dimer of each of the four histone proteins (H2A, H2B, H3 and H4) (Thomas, 1984). Each histone has a globular protein ‘core’ domain as well as an unstructured “tail” at the N-terminus, which can undergo many post-translational modifications (PTMs) (Serra et al., 1983; Van Holde et al., 1974). Histone PTMs can signal the chromatin to open up to initiate gene expression, or they may act compact or close the DNA for gene silencing (Soffer, 1973). Various PTMs can also act as a signaling pathway that recruit specific proteins to their chromatin substrates (Schlick et al., 2012). There are several known histone PTMs, but the most prevalent are phosphorylation, acetylation, methylation and ubiquitination (Walsh et al., 2005). Proteins that interact with these epigenetic modifications are grouped into three categories; “writers”, “erasers” or “readers”. “Writers” such as histone acetyltransferases (HATs) produce acetylation modifications on the nucleosome, while protein recognition modules such as the bromodomain (BRD) “readers” will bind to these acetyllysine modifications (Janzen et al., 2010). These PTMs aren’t permanent however, since “erasers” such as histone deacetylases (HDACs) are able to remove the acetylation PTM (Janzen et al., 2010). In the past 15 years over 15 histone reader domains have been described, these include the PWWP domain, chromodomains, ADD domains, PHD fingers and bromodomains (Kim et al., 2016). PHD fingers were discovered in 1993, and are known to bind histone H3 tri-methylated at lysine 4 (H3K4me3), the unmodified histone H3 tail as well as certain acetyllysine modifications (Aasland et al., 1995; Wysocka et al., 2006). In 1999, BRDs were discovered as the first domain to exclusively bind acetylated lysine (Dhalluin et al., 1999).

There are approximately 42 known human BRD-containing proteins, each of which posses between one and six BRDs (Schultz et al., 2000). Bromodomains are able to bind multiple acetylation marks from all four histone proteins, and shortly after their discovery Scanlan et al., revealed that one bromodomain-containing protein (bromodomain testis associated, BRDT) was linked to the development of cancer (Dhalluin et al., 1999; Scanlan et al., 2000; Winston and Allis, 1999). BRD-containing proteins are divided into eight sub-families based on their sequence and structural similarities (Filippakopoulos et al., 2010). All BRDs have a distinct secondary structure of a left-handed four alpha-helix bundle connected by two loops (ZA and BC) (Sanchez and Zhou, 2009). The ZA and BC loops form a hydrophobic pocket that coordinate the acetylated lysine on the histone tail (Sanchez and Zhou, 2009). Despite

their conserved structural fold, the sequence similarity between each of the eight bromodomain sub-families is drastically different, especially within the ZA and BC loop regions (Sanchez and Zhou, 2009). The most conserved residues are found in the acetyllysine binding pocket, and include Tyr1125, Tyr1167 and Asn1168 (Sanchez and Zhou, 2009). The BET (bromodomain and extra terminal) family (sub-family II) has been the main focus of BRD research due to their association with transcriptional regulators linked to c-MYC in the pathogenesis of human cancers and their strong potential for druggability (Delmore et al., 2011; French et al., 2001; Vidler et al., 2012). In 2010 and 2011 two groundbreaking small molecule inhibitors were developed, JQ1 and I-BET, that were found to block family II bromodomain recognition of acetyllysine modifications (Dawson et al., 2011; Filippakopoulos et al., 2010). JQ1 has been shown to inhibit cell growth in ovarian cancer, medulloblastoma and NUT midline carcinoma (Grayson et al., 2014; Qiu et al., 2015; Venkataraman et al., 2014). While I-BET was proven to be an effective treatment for mixed lineage leukemia (MLL) (Dawson et al., 2011). These proof of concept studies opened the door to the therapeutic potential of other bromodomain sub-families, and directly influenced a computational analysis of all eight BRD sub-families for their potential druggability (Vidler et al., 2012). While the BET family was predicted to have the highest druggability, all other sub-families showed potential as drug targets, particularly families I and IV (Vidler et al., 2012). (For a more in-depth look at BRD inhibitors refer to (Filippakopoulos and Knapp, 2014)).

Research on the family IV BRD-containing proteins (BRPF1, BRPF2, BRPF3, BRD7, BRD9, ATAD2 and ATAD2b) has been limited since investigations into the therapeutic potential of bromodomain inhibitors has mainly focused on the BET bromodomain family. However, recent studies on the family IV bromodomain proteins have linked them to many types of cancer. This review will summarize what is currently known about the biological roles of family IV bromodomains, the histone modifications they recognize, and their link to disease.

I. Bromodomain and PHD finger (BRPF) containing proteins

The bromodomain and PHD finger containing (BRPF) proteins (BRPF1/2/3) are composed of a bromodomain (BRD) and a plant homeodomain (PHD) (Qin et al., 2011). BRPF1 is a component of the MOZ/MORF (also known as KAT6a/KAT6b) histone acetyltransferase complexes (HATs), which are known to acetylate histones H2A, H2B, H3 and H4 (Figure 1A) (Champagne et al., 2001; Holbert et al., 2007; Kitabayashi et al., 2001). Both BRPF2 and BRPF3 are subunits in the HBO1 HAT, which acetylates histone H4 at lysine 5, 8 and 12 (Figure 1B) (Avvakumov and Cote, 2007). The MOZ/MORF and HBO1 complexes contain both reader and writer domains and play a role in disease progression. The MOZ HAT has been associated with a poor prognosis in a subtype of acute myeloid leukemia (AML) due to its involvement in chromosomal translocations fusing it to the p300, CBP and TIF2 proteins (Borrow et al., 1996; Brown et al., 2012; LaMorte et al., 1998; Zhong et al., 2000). HBO1 is overly expressed in testicular tumors, breast adenocarcinomas, and ovarian serous carcinomas (Iizuka et al., 2009; LaMorte et al., 1998).

The BRPF proteins all contain a double PHD domain separated by a mononuclear zinc (Zn) knuckle, known as the PZP domain (Perry, 2006). The physiological role of this motif is unknown, however, it preferentially recognizes the unmodified histone H3, and methylation of lysine 4 (H3K4me1/2/3) weakens the histone interaction (Lalonde et al., 2013). In BRPF2, the PHD2 region of the PZP domain was shown to interact with DNA, and the PZP domain of BPRF1 was later shown to interact simultaneously with the unmodified histone H3 tail and nucleosomal DNA through PHD1 and PHD2, respectively (Klein et al., 2016). However, the function of the PZP domain in BRPF3 has yet to be determined. In addition to the PZP domain the BRPF1/2/3 proteins contain a C-terminal bromodomain, which are highly conserved and share 56.3% sequence identity and 91.5% similarity. The BPRF1/2/3 proteins act as a scaffold for the formation of the MOZ/MORF and HBO1 HAT complexes and the action of multiple chromatin reader domains within these HATs are thought to be important for recruiting their acetylation activity to chromatin to up-regulate gene expression (Carlson and Glass, 2014).

BRPF1

The human BRPF1 protein (also known as Peregrin) is a subunit of the MOZ HAT, and based on its PHD finger and bromodomain it was postulated to be involved in chromatin remodeling (Thompson et al., 1994). BRPF1 anchors the MOZ catalytic subunit to the hEaf6 and ING5 subunits to stimulate the complex's acetyltransferase activity (Ullah et al., 2008) (Figure 1A). The BRPF1 bromodomain was shown to preferentially recognize histones H2AK5ac (K_d = 48.5 ± 1.5μM), H4K12ac (K_d = 86.5 ± 9.1μM), H3K14ac (K_d = 626 ± 27μM), H4K8ac (K_d = 697 ± 57μM) and H4K5ac (K_d = 1236 ± 327μM) (Table 1) (Poplawski et al., 2014). Recently, family IV bromodomains were also shown to interact with non-acetyl acyl post-translational modifications that are found with low frequency on the lysine residues of histone tails. Thus, in addition to recognizing acetylated lysines, BRPF1 also demonstrated the ability to recognize propionylated lysine on histone H4 that was di-propionylated at lysine 5 and lysine 8 (H4K5prK8pr) (Flynn et al., 2015). Larger acyl groups such as butyrylation and crotonylation were tested, but no binding interaction was observed, and the binding affinities for these interactions were not reported (Flynn et al., 2015). The MOZ HAT acetylates the histones once it is bound to chromatin (Kitabayashi et al., 2001). Acetylation of the chromatin would likely increase the affinity of the MOZ HAT complex as the bromodomain would recognize the newly created acetyllation marks (Carlson and Glass, 2014). This creates a positive feedback loop where activation of the MOZ HAT results in recruitment and stabilization of more MOZ on the chromatin, and propagates an increase of acetylation modifications locally, resulting in the up-regulation of gene expression (Carlson and Glass, 2014).

An extensive network of hydrogen bond and hydrophobic interactions are involved in coordinating the histone H2AK5ac and H4K12ac ligands (PDBIDs 4QYL and 4QYd) to the BRPF1 bromodomain (Lubula et al., 2014a). The carbonyl oxygen of the N-acetyl group on acetylated histone ligands forms a hydrogen bond interaction with the amide nitrogen of the conserved Asn83 residue deep in the hydrophobic binding pocket of the BRPF1 bromodomain (Figure 2A) (Lubula et al., 2014a; Lubula et al., 2014b). Figure 2A shows that the acetylated histone ligands are also stabilized by hydrogen bond contacts through ordered

water molecules in the binding pocket. Tyr40 is especially important for coordination of the carbonyl oxygen on the acetyllysine moiety via a water molecule (Lubula et al., 2014a). Water also mediates the hydrogen bond interaction between the ε-amino group and the backbone carbonyl of Ile27 (Lubula et al., 2014a). The 'WPF shelf' motif in BET bromodomains is important for formation of the acetyllysine binding pocket floor, and in BRPF1 these residues consist of Asn26, Ile27 and Phe28 (Orange, Figure 2A and 2B). Another important feature of acetyllysine coordination is the presence of a "gatekeeper" residue (usually Val or Ile) that partially blocks the hydrophobic region deep in the binding pocket allowing it to bind to both the acetyl group and aliphatic part of the acetyllysine (Chung et al., 2011; Flynn et al., 2015). In BRPF1 the gatekeeper residue was discovered to be Phe89 (Magenta, Figure 2A and 2B), which interestingly exhibits different orientations of the phenyl ring depending on which acetylated histone ligand was bound (Zhu and Caflisch, 2016). In 2015 Tallant C, et al., deposited a crystal structure of the BRPF1 bromodomain in complex with the di-acetylated histone ligand H4K5acK8ac (PDBID 5FFW), in which each acetylated lysine residue was bound to a separate bromodomain module. Coordination of the di-acetylated peptide is conserved with the binding mode of singly-acetylated lysine groups, however this structure demonstrates that it is physiologically possible for two bromodomains to bind to adjacent acetyllysine modifications simultaneously on the same histone tail.

BRPF2 and BRPF3

BRPF2 (also known as BRD1) and BRPF3 both contain the PZP motif and a bromodomain, but may play different roles in disease. BRPF2 has been linked to schizophrenia and bipolar disorder in some populations by way of a small-nucleotide polymorphism (SNP) (Nyegaard et al., 2010). BRPF3's molecular and biological function is currently unknown, however, it appears to be expendable in mice, whereas removal of BRPF2 was demonstrated to be lethal in mice (Yan et al., 2016). Both proteins preferentially join the quaternary complex of HBO1 HAT, along with the ING4 and hEaf6 subunits (Demont et al., 2014). HBO1 acts as a histone H4 acetyltransferase that is crucial in transcription and DNA replication licensing (Burke et al., 2001). Currently, there is limited information on the histone ligands of the BRPF2 and BRPF3 bromodomains. As with BRPF1, BRPF2 is able to bind the histone H4K5acK8ac modification with an affinity of 156 ± 3 μM by ITC, and binding was detected between the BRPF2 bromodomain and the di-propionylated histone ligand H4K5prK8pr (Flynn et al., 2015). The BRPF3 bromodomain also binds to di-acetylated and di-propionylated histone ligands, but the affinity of these interactions was not reported (Flynn et al., 2015). Since the sequence similarity of the BRPF2/3 bromodomains to the BRPF1 bromodomain is high, they likely also recognize a similar subset of single-acetylated histone modifications, but this remains to be determined experimentally.

II. Bromodomain containing (BRD) proteins

Two of the family IV bromodomain-containing (BRD) proteins (BRD7/9) are part of the SWI/SNF (SWItch/Sucrose Non-Fermenting) chromatin-remodeling complexes, which regulates gene expression (Figure 1C) (Kaeser et al., 2008). BRD7 is a subunit in the polybromo-associated BRG1-associated factor (PBAF) complex, and it was shown that the mRNA expression level of BRD7 is down-regulated in both colorectal and nasopharyngeal

carcinomas (Kaeser et al., 2008; Wu et al., 2013; Zhou et al., 2004). BRD7, through its interaction with BRCA1, also regulates BRCA1-dependant transcription and thus is postulated to be a tumor suppressor (Harte et al., 2010). Little is known about the BRD9 protein in disease, but a recent study determined that it was required for the proliferation of AML (Hohmann et al., 2016). Despite their involvement in unrelated diseases, the BRD7 and BRD9's bromodomains are highly conserved with 71.8% identity and 88.7% similarity, and these bromodomains share the same "gatekeeper" residue in their acetyllysine binding pocket (Tyr106) (Crawford et al., 2016).

The BRD7 bromodomain is located near the N-terminus of the ~650 amino acid BRD7 protein. Along with being involved in gene regulation via the SWI/SNF complex, BRD7 also regulates p53 and PI3K activity (Chiu et al., 2014; Drost et al., 2010). BRD7's potential function as a tumor suppressor has generated a lot of interest in this protein, and in 2007 histone-binding experiments on the BRD7 bromodomain were conducted. The results demonstrated that BRD7 has weak binding affinity to five acetylated histone ligands including; H3K9ac (K_d = 3.96 ± 0.44 mM), H3K14ac (K_d = 1.19 ± 0.02 mM), H4K8ac (K_d = 1.79 ± 0.10 mM), H4K12ac (K_d = 3.42 ± 0.14 mM) and H4K16ac (K_d = 2.56 ± 0.11 mM) (Table 1) (Sun et al., 2007).

The biological function of BRD9 has yet to be elucidated, however, the ~600 amino acid bromodomain-containing protein has been connected to many different cancer types (Picaud et al., 2015). Interestingly, the bromodomain of BRD9 is mutated in uterine corpus endometrial carcinoma, lung squamous cell carcinoma and prostate adenocarcinoma (Barbieri et al., 2012; Cancer Genome Atlas Research, 2012; Cancer Genome Atlas Research et al., 2013). BRD9 malfunction can also be linked to non-small-cell lung cancer, cervical cancer and hepatocellular carcinoma (Cleary et al., 2013; Kang et al., 2008; Scotto et al., 2008). Although less is known about the biological ligands of this bromodomain it has been shown to recognize the doubly acetylated histone H4K5acK8ac (K_d = 64 ± 5 μM) (Table 1) (Flynn et al., 2015). Like BRPF1/2/3, BRD9 and BRD7 are able to recognize the di-propionylated ligand H4K5prK8pr, but they are the only family IV bromodomains that are also able to accommodate the larger butyryllated lysine side chain in histone H4K5buK8bu (Flynn et al., 2015). Interestingly, the BRD9 bromodomain binds to the H4K5buK8bu ligand with a similar affinity to the di-acetylated histone ligand with a K_d of 60 ± 10 μM. However, binding to larger acyl chains was not tolerated, and the di-cryotonyllated histone ligand H4K5crK8cr showed no binding in ITC experiments (Flynn et al., 2015). Two key amino acid residues are important for coordination of the butyryllated histone ligand, as mutation of either the Tyr106 "gatekeeper", or the core Met92 residue resulted in no binding of the BRD9 bromodomain with this unique di-butyryllated H4K5buK8bu ligand (Flynn et al., 2015). Also, mobility in the phenylalanine residue Phe45 in the 'WPF shelf' motif appears to allow expansion of the acetyllysine binding pocket as the size of the acyl modification increased up to the butyryl group (Figure 2C). However, no further movement of Phe45 was detected with binding of the crotonyllated lysine, suggesting that the size of this larger acyl modification could not be accommodated by the binding pocket (Flynn et al., 2015). In the deposited crystal structures of BRD9 in complex with the di-acetylated, di-butyryllated and di-crotonyllated ligands each histone peptide is bound by two monomers of a BRD9 bromodomain (PDBIDs 4YYI, 4YYJ and 4YYK), with one

monomer binding to the modified lysine 5, and the other binding to the modified lysine 8 (Figure 2D). This unique mode of histone recognition suggests that dimerization of bromodomains may be important for recruitment of chromatin remodeling complexes to regulate gene expression, and indicates that di-acetylated or di-acylated regions on histone H4 may have alternate epigenetic signaling functions than singly acetylated regions of the chromatin.

III. ATPase family, AAA domain containing 2 (ATAD2) proteins

The ATPase family, AAA domain containing 2 (ATAD2) proteins (ATAD2/ATAD2b) each contain two conserved domains: an N-terminal AAA+ ATPase domain and a C-terminal bromodomain (Ciro et al., 2009; Leachman et al., 2010). The ATPase Associated with diverse cellular Activities (AAA+) domain is composed of two alpha helical subdomains that are responsible for nucleotide hydrolysis of ATP by nucleophilic attack on the gamma-phosphate (Hanson and Whiteheart, 2005). Proteins in the AAA+ superfamily often assemble into hexameric ring complexes and function in a wide range of cellular processes that require the remodeling of macromolecules (Snider et al., 2008). In ATAD2, the AAA+ domain was shown to be important for oligomerization of the protein, and also for recognition of acetylated histones (Caron et al., 2010; Koo et al., 2016). ATAD2 is considered a critical transcription factor in malignant cells and up-regulates anti-apoptotic activity in both prostate and breast cancer cells (Altintas et al., 2012; Boussouar et al., 2013). ATAD2 has become an exciting new epigenetic target because of its over-expression and association with multiple cancers, which now also includes ovarian and endometrial carcinoma, as well as gastric, colorectal and cervical cancer (Krakstad et al., 2015; Luo et al., 2015; Wan et al., 2014; Zhang et al., 2015; Zheng et al., 2015). *ATAD2b* is a poorly studied paralog of the *ATAD2* gene, and although the ATAD2 and ATAD2b proteins are highly conserved, there is little known about the function of ATAD2b or its role in oncogenesis. Interestingly, these two proteins appear to have different expression patterns. ATAD2 is predominantly found in reproductive tissue, while ATAD2b appears to be primarily located in neurological tissue (Caron et al., 2010; Ciro et al., 2009; Kalashnikova et al., 2010; Leachman et al., 2010; Zou et al., 2009). Despite their differences in expression, the ATAD2/b bromodomains are highly homologous with 74.7% amino acid sequence identity and 94.4% similarity, suggesting they may have redundant functions (Leachman et al., 2010).

ATAD2

The first reported molecular function of the ATAD2 protein (also known as ANCCA) was as a co-activator of the estrogen and androgen receptors (Figure 1D) (Zou et al., 2009; Zou et al., 2007). Subsequently, ATAD2 was also reported to be a co-factor involved in stimulating MYC and E2F-dependent cell proliferation (Ciro et al., 2009; Revenko et al., 2010). The AAA+ ATPase domain is required by ATAD2 for MYC transcriptional activation, whereas both the AAA+ ATPase and bromodomain are required for its function as an estrogen and androgen receptor co-activator (Revenko et al., 2010; Zou et al., 2009). ATAD2 was also recently reported to function as a generalist enhancer of chromatin dynamics that promotes cellular differentiation and proliferation (Morozumi et al., 2015). Significantly, ATAD2 was

shown to be tightly associated with nucleosomes and enriched on actively replicating chromatin (Morozumi et al., 2015), which is strongly correlated to the recent discovery that ATAD2 may play a crucial role in regulating heterochromatin replication and the formation of higher order chromatin structures (Koo et al., 2016). The bromodomain of ATAD2 is able to recognize acetylated histones including; H4K5ac (K_d = 22 μM) (Poncet-Montange et al., 2015), H4K12ac (K_d = 2.5 μM) (Koo et al., 2016) and the di-acetylated histone H4K5acK12ac (no binding data) (Koo et al., 2016) (Table 1). ATAD2 was also shown to bind to histone H3K14ac, but this ligand was later determined to make contact with a site outside the acetyllysine-binding pocket of the ATAD2 bromodomain (Duan et al., 2013; Poncet-Montange et al., 2015). As with the other family IV bromodomains both the ATAD2 and ATAD2b bromodomains were also able to bind propionylated lysine on histone H4 in the peptide screening array carried out by Flynn, et al., 2015, but they could not recognize larger acyl groups.

Contacts critical for histone ligand binding have been identified based on structures of the ATAD2 bromodomain solved in complex with histone H4K5ac (PDB ID: 4TT2) and H4K12ac (PDB ID: 4QUT) (Morozumi et al., 2015; Poncet-Montange et al., 2015). For both histone ligands, the acetylated-lysine is coordinated by a conserved asparagine residue (Asn1064) and Tyr1021, which makes a water-mediated hydrogen bond to coordinate the acetyllysine carbonyl oxygen (Figure 2E). Two regions of the bromodomain-binding pocket are known to be important for guiding ligand binding selectivity: the ‘gatekeeper’ residue and the ‘WPF shelf’ motif (Romero et al., 2016). Residues Arg1007, Val1008 and Phe1009 make up the ‘RVF’ shelf of ATAD2, while the gatekeeper residue is Ile1074 (Figure 2E and 2F) (Romero et al., 2016). In BRPF1 and BRD7/9 the gatekeeper residue is a phenylalanine and a tyrosine, respectively, and these larger aromatic residues may be responsible for driving the differences in histone ligand selectivity observed among the family IV bromodomains (Table 1).

The connection between the epigenetic function of ATAD2 and cancer also suggests it may be viable candidate as a therapeutic target. Overexpression of ATAD2 has been linked to poor prognosis in cancer patients (Caron et al., 2010; Kalashnikova et al., 2010), and development of specific ATAD2 inhibitors may be a possible means of improving patient outcomes. Since the discovery of small molecule bromodomain inhibitors such as JQ1 and I-BET that selectively inhibit bromodomains by directly targeting the acetyllysine binding site with nanomolar affinity (Filippakopoulos et al., 2010; Nicodeme et al., 2010), the development of new bromodomain inhibitors has become an intense area of investigation for the treatment of multiple disease types (Filippakopoulos and Knapp, 2014; Papavassiliou and Papavassiliou, 2014). First generation bromodomain inhibitors for ATAD2 have been developed (Bamborough et al., 2016b; Bamborough et al., 2015; Poncet-Montange et al., 2015). The first compounds generated by the MD Anderson Cancer Center (compounds 1/2) bound to the ATAD2 bromodomain in the micromolar range (K_d = 175–202 μM) (Poncet-Montange et al., 2015). Subsequent efforts by GlaxoSmithKline produced tool compounds (compound 38 and 42), which have sub-100 nanomolar binding affinity and greater than 100-fold binding selectivity over the BET bromodomain family (Bamborough et al., 2015). Generation of these ATAD2 inhibitors provide new avenues for investigation of the

biological role of the ATAD2 bromodomain in normal and disease states, and serve as starting points for the development of novel therapeutics.

ATAD2b

ATAD2b (KIAA1240), is a poorly studied paralog of the ATAD2 gene, and although ATAD2 and ATAD2b are highly conserved (94.4% amino acid similarity), there is little known about the function of ATAD2b or its role in oncogenesis. A 2010 study by Leachman et al., provided the first insights into the function of the ATAD2b protein. They demonstrated that ATAD2b is expressed predominantly in neurological tissues, including the spinal cord grey matter and dorsal root ganglia of chicken embryos (Leachman et al., 2010). ATAD2b was also found expressed in human tumors including glioblastoma, oligodenroglima and breast carcinoma (Leachman et al., 2010). Thus, they hypothesized that the function of ATAD2b protein may to be linked to neuronal differentiation as well as tumor progression (Leachman et al., 2010). At this time the apo-structure of ATAD2b bromodomain has been solved (PDB ID: 3LXJ), but the histone ligands it recognizes are currently unknown. More research is needed to determine if the biological role of the ATAD2b-bromodomain containing protein is independent or overlapping with the function of ATAD2. This information will be crucial for the development of next generation ATAD2 bromodomain inhibitors, which will need to posses increased selectivity over other family IV bromodomains.

Conclusions

The connection between the epigenetic function of bromodomain-containing proteins and cancer suggests that they may be viable candidates for drug targeting in cancer treatment. Breakthrough studies by Filippakopoulos et al., 2010 and Nicodeme et al., 2010 identified small molecule ligands that can bind to the BET family of bromodomains with very high affinity and specificity. Family IV bromodomains are one of the lesser-known bromodomain sub-families, but evidence points to their potential for the development of novel drugs to combat many types of cancers (Vidler et al., 2012). To date there has been some success in directly targeting the family IV bromodomains with small molecule inhibitors including the BRPF1 inhibitor GSK6853 (Bamborough et al., 2016a), the dual TRIM24/BRPF1 inhibitor IACS-9571 (Palmer et al., 2015), the BRD7/9 inhibitor LP99 (Clark et al., 2015) and the ATAD2 inhibitors C-42 (Bamborough et al., 2015) and GSK8814 (Bamborough et al., 2016b). To gain more information about the potential of family IV bromodomains as therapeutic targets it will be crucial to characterize their physiological functions and the contribution of each bromodomain-containing protein to the development of disease. Also, even though many of the apo structures of these bromodomains are solved, their histone targets and how they recognize them is still largely uncharacterized. For instance, no histone binding data is available on the BRPF3 or ATAD2b bromodomains, and only limited information is known for the BRPF2, BRD9 and ATAD2 bromodomains (Table 1). Interestingly, the current data suggests that the BRPF1/2/3, BRD7/9 and ATAD2/b bromodomains each use different binding modes to recognize and select for their histone ligands. Further studies are needed to elucidate the mechanism of histone recognition used by each these bromodomains in order to develop more specific, second generation inhibitors, that do not have overlapping activity within the family IV bromodomain subfamily. The

currently available chemical probes will allow for continued investigation on the epigenetic signaling pathways modulated by family IV bromodomains, and hold much promise for discerning their roles in normal and pathological gene expression, as well as their therapeutic potential.

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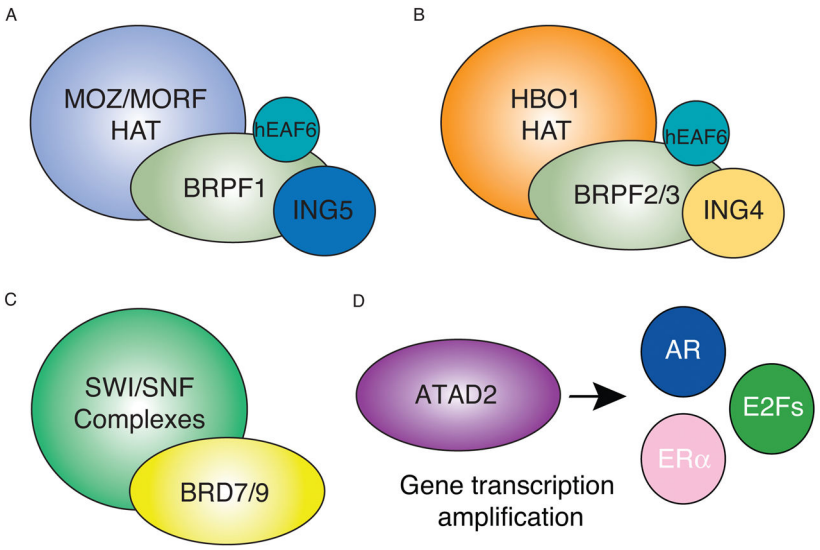

Figure 1.

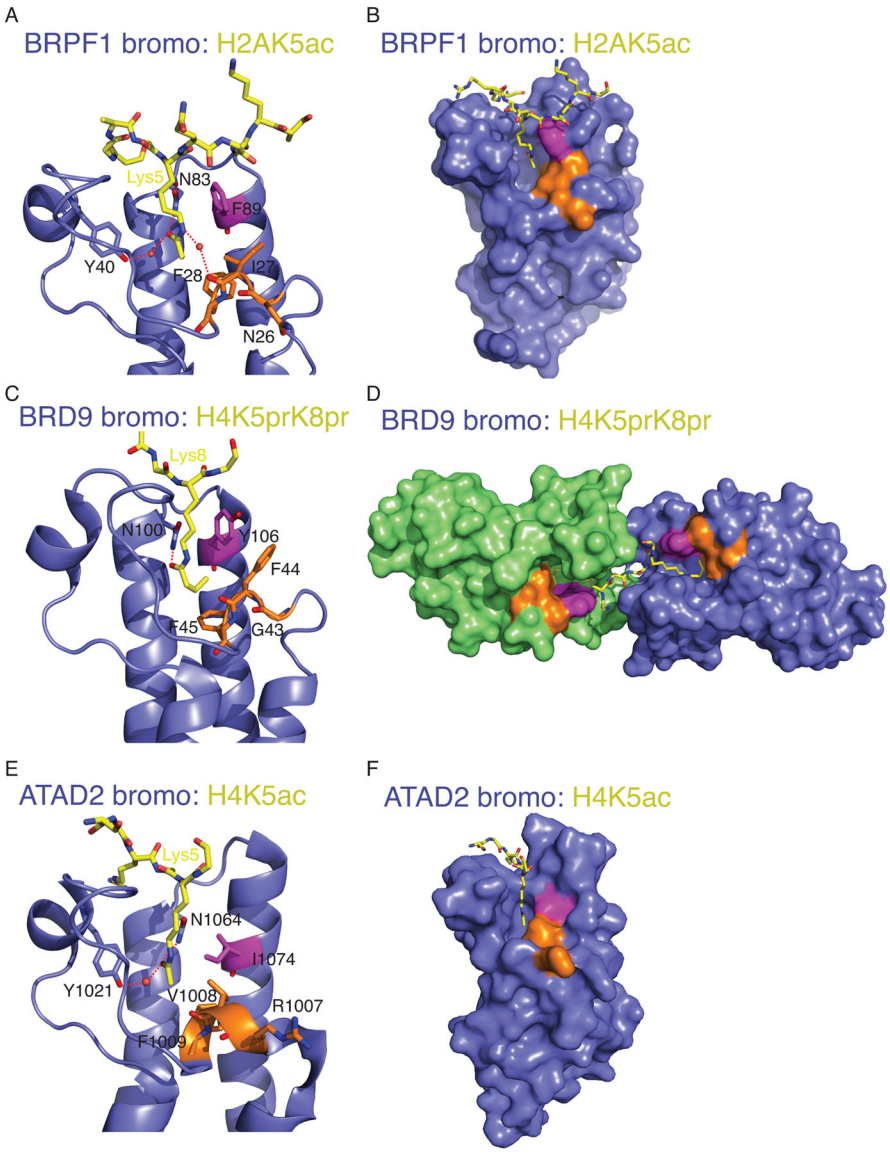

Figure 2.

Table 1

Binding of family IV bromodomains to histone ligands.

Protein	H2AK5ac	H3K9ac	H3K14ac	H4K5ac	H4K8ac	H4K12ac	H4K16ac	H4K5acK8ac	H4K5prK8pr	H4K5buK8bu	H4K5acK12ac
BRPF1	48.5 ± 1.5 μM	NB	626 ± 27 μM	1236 ± 327 μM	697 ± 57 μM	86.5 ± 9.1 μM	ND	ND	*	NB	ND
BRPF2 (BRD1)	ND	ND	ND	ND	ND	ND	ND	156 ± 3 μM	*	NB	ND
BRPF3	ND	ND	ND	ND	ND	ND	ND	ND	*	NB	ND
BRD7	ND	3.96 ± 0.44 mM	1.19 ± 0.02 mM	ND	1.79 ± 0.10 mM	3.42 ± 0.14 mM	2.56 ± 0.11 mM	ND	*	*	ND
BRD9	ND	ND	ND	ND	ND	ND	ND	64 ± 5 μM	44 ± 3 μM	60 ± 10 μM	ND
ATAD2	ND	ND	*	22 μM	ND	2.5 μM	ND	ND	*	NB	*
ATAD2b	ND	ND	ND	ND	ND	ND	ND	ND	*	NB	ND

*Binding interaction demonstrated, but no affinity reported

Lysine modifications and abbreviations are: acetyl (Kac), propionyl (Kpr) and butyryl (Kbu)

NB = No binding

ND = Binding not determined