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Bromodomains as therapeutic targets in cancer

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Abstract
The malleability of the epigenome has long been recognized as a unique opportunity for therapeutic intervention. Interest in targeting components of the epigenetic machinery for therapeutic gain had initially been aimed at chromatin modifying enzymes. However, advances in medicinal chemistry have now made it possible to exploit protein–protein interactions at the chromatin interface. Bromodomains (BRD) are a conserved motif used by a large number of chromatin-associated proteins to recognize and bind acetylated histone tails. Small molecules with high specificity for the Bromodomain and Extra Terminal family of proteins (BRD2, BRD3, BRD4 and BRDT) have recently been shown to have remarkable pre-clinical efficacy in various malignancies. These findings have provided the impetus for exploring other BRD proteins as novel targets in cancer therapy.

Keywords: *bromodomain; BRD4; cancer; epigenetics*

INTRODUCTION

The dawn of the genomic era has focused our attention on the central role of epigenetic regulators in cancer biology. It is now apparent that many of the components involved in the epigenetic regulation of gene expression, DNA repair and replication are commonly mutated in various cancers [1,2]. Importantly, the plasticity of the epigenome lends itself well to therapeutic manipulation and as such there is now great interest in generating small molecules that are effective in modulating the activities of the various protagonists involved in controlling the epigenetic landscape.

A fundamental tenet in chromatin biology is that all components of the nucleosome are covalently modified and these modifications are dynamic entities that play an instructive role in various biological processes. Currently, there are four different DNA modifications and over 16 separate histone modifications described [2]. These modifications serve two main functions; they alter chromatin structure by enhancing or weakening the non-covalent interactions between histone–histone or histone–DNA and they provide an informative platform for the dynamic recruitment of chromatin modifying/remodelling enzymes that then establish local and global chromatin patterns. Whilst many of the proteins that control and interpret these modifications have been implicated in oncogenesis the subject of this review will focus on the bromodomain (BRD) proteins that bind acetylated lysine residues on histones. For a more comprehensive overview on cancer epigenetics, the reader is referred to several recent reviews [1–5].

Histone acetylation is one of the best-studied histone modifications. It has been implicated in the control of gene expression, DNA repair and replication. Levels of acetylation are controlled by the competing activities of two enzymatic families: the histone acetyl transferases (HATs) and the histone

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deacetylases (HDACs). HATs catalyse the transfer of an acetyl group to the ε-amino group of lysine side chains of a histone protein, whereas HDACs' function to remove it. Altered levels of histone acetylation are widely reported in various malignancies [6] and the pharmacological interference with the acetylation levels represents a promising approach for cancer therapy. A widely cited proof of principle for this approach is HDAC inhibitors which inactivate many of the enzymes that remove acetyl groups from acetylated lysines within histone and non-histone proteins [7]. In fact, HDAC inhibitors are currently used for the treatment of cutaneous T-cell lymphoma and are being assessed in clinical trials for the treatment of several other types of malignancies [8–10]. The addition of an acetyl group on histones serves two main purposes: first, it neutralizes the positive charge on lysine thus reducing the electrostatic interaction with the negatively charged DNA, relaxing the chromatin structure and facilitating the access of various chromatin associated proteins; second, it provides a binding site for proteins with specialized binding motifs that recognize this histone modification. The best characterized binding motif for lysine acetylation is the BRD.

BROMODOMAINS: STRUCTURE AND FUNCTION

BRDs are 110 amino acid modules that are highly conserved throughout evolution. They were first identified in the protein encoded by the *D. melanogaster brahama* gene. The BRD-containing protein family includes various transcriptional co-regulators, chromatin modifying enzymes and nuclear scaffold proteins that are able to specifically recognize acetylated lysine residues on histone tails. BRD containing proteins can also bind acetylated lysine residues on non-histone proteins. For instance, it has been reported that BRD4 can bind acetylated lysines on the NF-kappaB subunit RelA [11] and similarly BRD3 has been shown to associate with acetylated GATA1 [12].

The BRD structure consists of a left-handed bundle of four antiparallel alpha helices linked by two loop regions (Figure 1). The co-crystal structures of BRDs bound to acetyl-lysine containing peptides show that the acetylated lysine is first recognized in a hydrophobic pocket located between the two loops, which is formed by the most highly conserved residues. This includes an asparagine at the core of the binding site, which engages the acetyl-lysine via a hydrogen bond between its NH_2 group and the acetyl carbonyl oxygen atom of the acetylated lysine. At the entrance of the binding pocket, residues located in the two loop regions interact with residues adjacent to the acetylated lysine in the target sequence, thus further reinforcing the binding through hydrophobic and electrostatic interactions. Although the acetyl-lysine binding pocket for all BRDs is hydrophobic, there is considerable variation in the electrostatic interactions at the opening of the pocket among BRD families [13]. This variation determines the specificity of individual BRDs and provides the opportunity to both sub-classify BRD proteins into families and develop specific small molecules that are specifically targeted against certain families.

The human genome encodes 46 diverse proteins that contain a total of 61 BRDs structurally clustered into eight distinct subfamilies (Figure 2). The first subfamily is comprised of a functionally diverse group of proteins that includes the HAT P300/CBP-associated factor (PCAF). Indeed, it was the

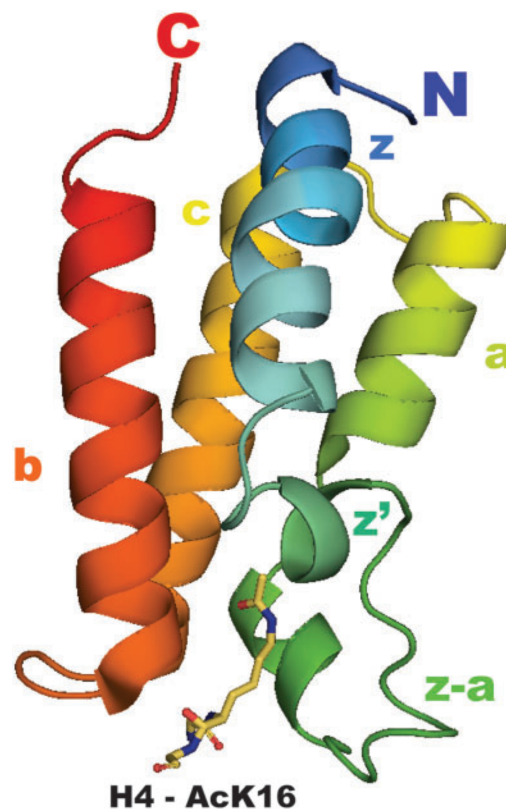

Figure 1: BRD4 bromodomain 1 structure showing the binding with the acetylated Lysine 16 on the tail of Histone H4.

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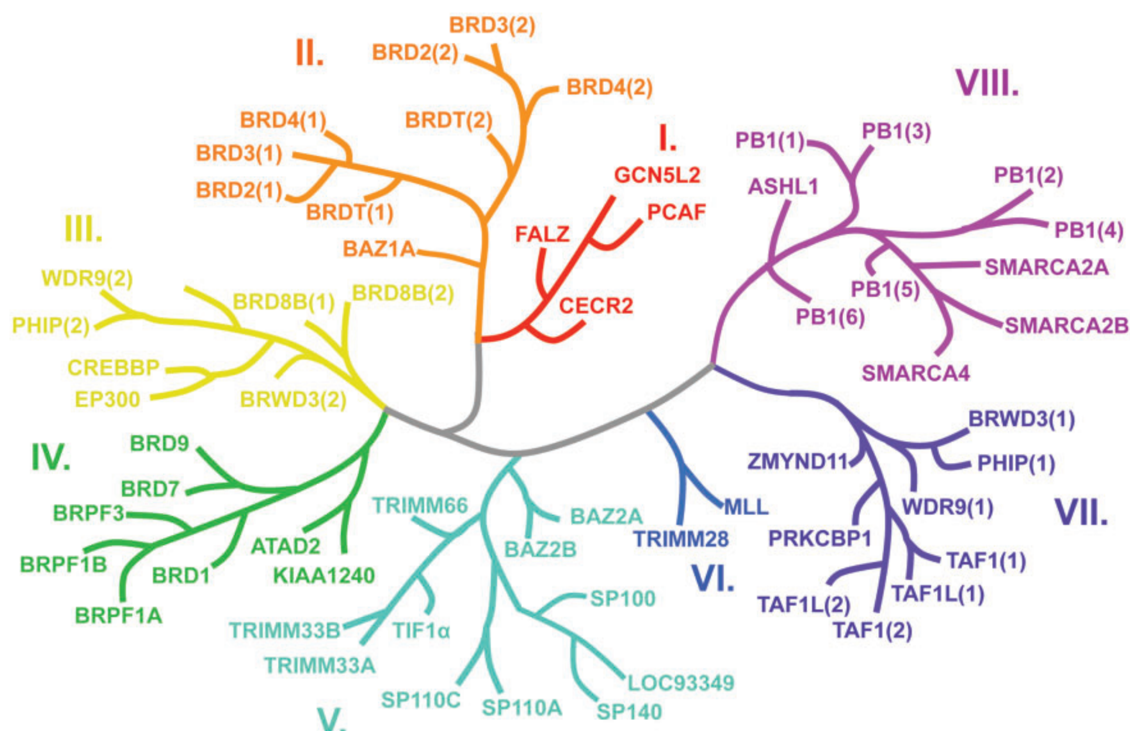

Figure 2: Phylogenetic tree based on the structure of the human BRDs. The BRDs are grouped in eight families, each family is identified by a roman number. In cases where several bromodomains are present on a single protein they are differentiated by the number in brackets. Adapted from Filippakopoulos *et al.* [13].

solution of the BRD structure of PCAF that demonstrated the ability of BRDs to bind to acetylated lysine's [14]. The Bromodomain and Extra Terminal (BET) proteins are grouped in the second subfamily and highly specific small molecule inhibitors of this family have recently emerged as promising therapeutic agents in inflammation and cancer [15]. Proteins within the fifth subfamily are structurally characterized by the presence of a methyl-lysine reader domain, the plant homeodomain (PHD) finger in tandem with a BRD. This tandem epigenetic reader module is necessary for the binding to chromatin and highlights an important theme in chromatin biology: the multivalent engagement of histone modifications by epigenetic reader proteins that contain more than one reader domain. Whilst an exhaustive description of the structural diversity of the various BRD families is beyond the scope of this review the reader is directed to an excellent and comprehensive review of this subject [16].

From a functional perspective, most of the BRD containing proteins are integral members of larger multi-subunit chromatin complexes. These can be roughly grouped in three major categories: chromatin modifiers, chromatin readers and chromatin remodellers. The first group is composed of enzymes that possess the capacity to introduce modifications on chromatin, such as histone methylation (MLL) and histone acetylation (CREBBP/p300). The second group contains proteins that do not possess enzymatic activity on chromatin but can recruit general transcription factors or chromatin modifying complexes (BET). The third group is composed of proteins with ATPase activity able to remodel chromatin (SMARCA4).

In the histone acetyl-transferase complexes, the BRD component is generally responsible for anchoring the HATs complex to acetylated chromatin so allowing the spreading of acetylation on adjacent nucleosomes. Likewise, several chromatin remodelling complexes, which are ATP-dependent remodellers of DNA–histones interactions and nucleosome position, are recruited to their appropriate genomic location by the BRD proteins they contain. This localization then facilitates gene expression by improving access for the transcriptional machinery. BET proteins contain two BRDs at their amino terminal and an extra-terminal domain at their carboxyl terminal, which is also involved in protein–protein interactions. Whilst the tandem BRDs of the BET

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proteins localize them to acetylated histones, the ET domain interacts with various transcription factors or chromatin remodellers aiding the localization of these proteins to chromatin. It is also feasible that the two BRDs of the BET proteins bind multiple adjacent acetyl-lysine residues on the same nucleosome, on different nucleosomes or on adjacent non-nucleosomal proteins. The complete nuclear BET interactome has recently been defined and serves to highlight the broad functional diversity of these essential BRD proteins [17].

BROMODOMAINS AS THERAPEUTIC TARGETS

The importance of transcriptional dysregulation as a sentinel event in the initiation and maintenance of oncogenesis has been well established. A wealth of literature describes the role that various transcription factors play in this process. The difficulty has been establishing a therapeutic strategy that negates the aberrant transcriptional programmes driven by these transcription factors. The recognition that many epigenetic regulators are either directly or indirectly co-opted to facilitate these oncogenic gene-expression programmes has opened untapped avenues for cancer therapeutics. A major focus in this arena is the opportunity to develop and deploy small molecules against epigenetic reader domains such as BRDs. Many of the BRD proteins play an integral role in the regulation of transcription via a direct association with transcription factors and/or the localization of chromatin modifying complexes. A further testament to the influence these proteins have in the oncogenic process is the fact that many of these proteins are recurrently mutated or aberrantly expressed in various malignancies (Table 1).

The BRD/histone interaction can be blocked by small molecules directed to the acetylated lysine recognizing pocket. These small molecules mimic the acetyl-lysine head group and interact with the conserved residues, while their lipophilic part interacts with the hydrophobic side chains of the binding site's residues [57]. Early NMR-based screening studies identified several compounds that were capable of selectively disrupting the acetyl-lysine BRD interaction [58,59]. These findings established the principle of selective BRD inhibition and provided the impetus for the recent development of highly potent and selective inhibitors of the BET family of BRD proteins [17,28,60]. The stage is now set for the development of a range of structurally distinct medicinal compounds that selectively inhibit the functionally diverse set of BRD proteins. These inhibitors may offer a novel therapeutic paradigm in range of malignancies [15].

CHROMATIN READERS

The BRD proteins that lack intrinsic chromatin modifying potential primarily function as scaffolds for transcription factors or other chromatin regulating factors. Exemplars of this group are the BET BRD proteins, which are epigenetic readers that recognize acetylated lysine residues on histone tails. The family includes four different proteins, BRD2, BRD3, BRD4 and BRDT and is characterized by the presence of two distinct BRDs at the N-terminal end of the protein. It is notable that members of the BET family are recurrently mutated or aberrantly co-opted in several cancers. For instance, BRD3 and BRD4 are involved in the pathognomonic chromosomal translocations seen in NUT midline carcinoma (NMC) [61]. In addition, BET proteins are part of transcriptional complexes that are essential for the initiation and maintenance of certain leukaemias. They are also associated with viral oncoproteins, such as the HPV E2 protein [62]. The important function of the BET proteins in gene regulation and their involvement in various cancers suggested that inhibitors of the BET BRDs maybe promising anti-cancer agents [15]. This led to the development of three different BET inhibitors: I-BET762, JQI and I-BET151. These drugs have been shown to be highly specific to the BET BRDs. They are cell permeable and able to displace the BET proteins from acetylated histones in a cellular context.

Translocations involving the mixed leukaemia gene (MLL) are the initiating event in a range of acute myeloid and lymphoid leukaemias. These diseases often share a common mechanism of transformation and are almost uniformly refractory to conventional therapies. A common feature of these translocations is the fusion of MLL gene with genes that code for members of the super elongation complex (SEC). The central role of transcriptional elongation in the pathogenesis of MLL leukaemia is further highlighted by the MLL mediated interaction with the polymerase associated factor complex (PAFc). Importantly, the functional integrity of both the SEC and PAFc has been shown to be

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Table 1: Bromodomains involved in cancer

Protein	Name	Function	Mutation in cancer	Altered expression in cancer
ASHIL	Ashl (absent, small or homeotic)-like	H3K36 methyltransferase	Mutated in lung cancer [18]	
ATAD2	ATPase family, AAA domain containing 2	Transcriptional regulator		Over expressed in different tumours [19]
BAZIA	BRD adjacent to zinc finger domain, IA	Chromatin remodelling factor		
BAZIB	BRD adjacent to zinc finger domain, IB	Tyrosine kinase; chromatin remodelling factor; transcriptional regulator		Over expressed in adenocarcinoma [20]
BAZ2A	BRD adjacent to zinc finger domain, 2A	Transcriptional repressor [21]		
BAZ2B	BRD adjacent to zinc finger domain, 2B [22]	Unknown		
BRDI	BRD containing I	H3 acetyl transferase; transcriptional regulator	Disrupted in ALL [23]	
BRD2	BRD containing 2	Chromatin reader; transcriptional regulator [24]		B cells lymphomagenesis [25]
BRD3	BRD containing 3	Transcriptional regulator [24]	Disrupted in NMC [26]	Implicated in AML [17]
BRD4	BRD containing 4	Transcriptional regulator	Disrupted in NMC [27]	Implicated in AML [17,28]
BRD7	BRD containing 7	Transcriptional regulator; tumour suppressor		Downregulated in breast cancer [29]
BRD8	BRD containing 8	Transcriptional regulator		Probably involved in colorectal tumour progression [30]
BRD9	BRD-containing protein 9	May play a role in chromatin remodelling and regulation of transcription		
BRDT	BRD, testis-specific	Chromatin remodelling factor		Over expressed in lung cancer [31]
BRPFI	BRD and PHD finger containing I	Transcriptional activator [32]		
BRPF3	BRD and PHD finger containing 3	H3 acetyl transferase		
BRWD3	BRD and WD repeat domain containing 3	Cell morphology	Disrupted in B-cell chronic lymphocytic leukaemia [33]	
CECR2	Cat eye syndrome chromosome region, candidate 2	Chromatin remodeller [34]		
CREBBP	CREB-binding protein	HAT	Disrupted in acute myeloid leukaemia [35]; mutated in B-non-Hodgkin lymphoma [36]	
EP300	EIA-binding protein p300	HAT	Disrupted in acute myeloid leukaemia [35], colon and gastric adenocarcinomas [37]	
FALZ	Fetal Alzheimer antigen	Transcriptional regulator [35]		
GCN5L2	General control of amino-acid synthesis 5-like 2	HAT; Transcriptional activator [38]		
KIAAI240	KIAAI240 protein (ATPase family, AAA domain containing 2B)	Histone-binding factor		Probably involved in neural tumorigenesis [39]

(continued)

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Table 1: Continued

Protein	Name	Function	Mutation in cancer	Altered expression in cancer
MLL	Myeloid/lymphoid or mixed-lineage leukaemia	Histone methyltransferase	Disrupted in acute leukaemias [40]	
PBRM1	Polybromo 1	Chromatin remodelling factor	Mutated in renal carcinoma [41]	
PCAF	P300/CBP-associated factor	Histone acetyltransferase		
PHIP	Pleckstrin homology domain interacting protein	Insulin signalling protein		Expressed in myeloma and epidermoid carcinoma cell lines [42]
SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	Chromatin remodelling factor; transcriptional coactivator	Mutated in HNSCC	Reduced expression in hepatocellular carcinoma [43]
SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	Chromatin remodelling factor; transcriptional coactivator	Mutated in rhabdoid tumour predisposition syndrome [44], lung cancer [45], breast cancer, prostate cancer, pancreas cancer and HNSCC (multiple studies reviewed in [46] and [2])	
SP100	SP100 nuclear antigen	Transcriptional regulation; tumour suppressor		Downregulated in laryngeal cancer [47]
SP110	SP110 nuclear body protein	Transcriptional regulator		CNVs in desmoplastic melanoma and malignant peripheral nerve sheath tumour [48]
SP140	SP140 nuclear body protein	Transcriptional regulator		SNPs in chronic lymphocytic leukaemia [49]
SP140L	SP140 nuclear body protein-like	Unknown		
TAF1	TAF1 RNA polymerase II, TATA box-binding protein (TBP)-associated factor,	Transcriptional regulator		
TAF1L	TAF1 RNA polymerase II, TBP-associated factor, 210 kDa-like	Transcriptional regulator [50]		
TRIM24	TRIM-containing 24	Transcriptional coactivator	Mutated in thyroid papillary carcinoma [51]	
TRIM28	TRIM-containing 28	Transcriptional corepressor [52]		
TRIM33	TRIM-containing protein 33	E3 ubiquitin-protein ligase	Mutated in thyroid papillary carcinoma [51]	
TRIM66	TRIM-containing protein 66	Transcriptional repressor [53]		
WDR9	WD repeat-containing protein 9	Chromatin remodeller; transcriptional regulator [54]		
ZMYND8	Zinc finger, MYND-type containing 8	Transcriptional regulator		Over expressed in cervical carcinomas [55]
ZMYND11	Zinc finger, MYND-type containing 11	Transcriptional corepressor [56]		

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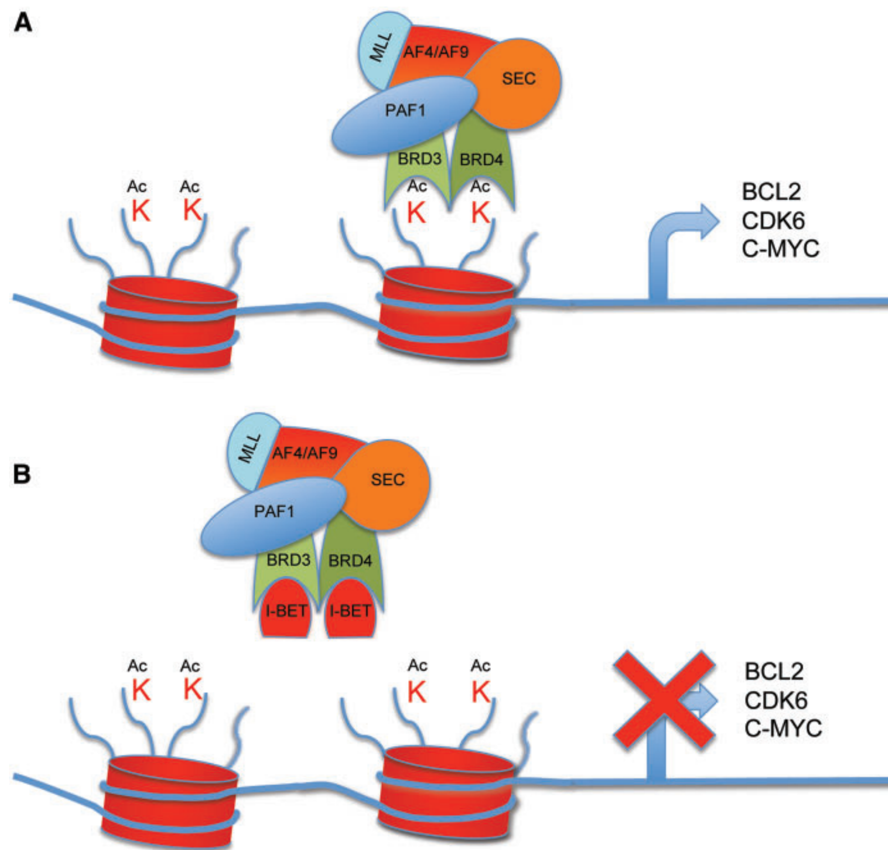

Figure 3: The BET proteins recruit the SEC and the PAFc on acetylated chromatin increasing transcriptional elongation (A). In the presence of the I-BET inhibitor, the BET proteins are displaced from acetylated chromatin and the productive transcription is reduced.

critical for the malignant transformation by MLL fusions [63,64]. Using a novel tripartite proteomic approach, Dawson *et al.* demonstrated that BRD3 and BRD4 are fundamental components of the PAFc and SEC complexes providing a therapeutic rationale for BET inhibitors in these leukaemia's [17] (Figure 3). Moreover, using a complementary approach, Zuber *et al.* showed that the downregulation of BRD4 by RNAi strongly reduced the viability of MLL-AF9 leukaemias both *in vitro* and *in vivo* [65]. Both groups went on to demonstrate that chemically distinct but equally selective inhibitors of the BET BRD proteins showed excellent efficacy in inducing growth arrest and apoptosis in MLL cell lines. This effect is achieved by the downregulation of pivotal oncogenes, such as MYC and BCL2 (Figure 3). These studies also showed the efficacy of BET inhibition in AML cell lines lacking MLL rearrangements, albeit at slightly higher concentrations to those seen in MLL-translocated leukaemias, suggesting that this therapeutic approach may be useful in different subtypes of AML and potentially in different haematopoietic malignancies [17,65]. Indeed, in concurrent studies two other groups showed that BET inhibition is effective in Multiple Myeloma and Burkitt's lymphoma [66,67]. Both these pathologies are characterized by an abnormal expression of MYC and the therapeutic effect shown by these studies was deemed to be mediated, at least in part, by the downregulation of MYC [66,67]. Together, these studies suggest MYC as one of the main target genes for BET inhibition. Nevertheless, the identification of the BET protein interactome shows that these proteins are involved in a large number of nuclear regulatory complexes suggesting that the therapeutic effect in different malignancies may be driven by a variety of diverse molecular mechanisms.

The tripartite motif (TRIM)-containing proteins are another family of epigenetic reader proteins that could be useful therapeutic targets in cancer. The TRIM proteins are characterized by the presence of a conserved N-terminal motif formed by a RING finger, one or two zinc-binding domains

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and a coiled-coil domain. These factors are involved in a range of homeostatic processes and have also been implicated in several diseases including cancer. The TRIM family is subdivided into 12 subgroups based on the C-terminal part of the protein. The TIF1 proteins, TRIM24 (TIF1a), TRIM 28 (TIF1b) and TRIM33 (TIF1g) constitute one of the 12 subgroups of the TRIM family and contain a PHD finger and a single BRD at their C-terminus. The presence of these epigenetic reader domains allows these factors to recognize and bind specific methylation/acetylation modifications on a single nucleosome and potentiate a specific transcriptional response.

TRIM24, the founding member of the TIF family, has been associated with the pathogenesis of several malignancies. The TRIM24-FGFR1 fusion is a rare but recurrent translocation observed in a subset of myeloproliferative diseases [68]. Other fusion proteins involving TRIM24 have also been observed in various solid cancers [51,69] and overexpression of TRIM24 is observed in subset of breast cancers where it confers a poor prognosis [70]. Experimental evidence in murine models of hepatocellular carcinoma has suggested a tumour suppressor function for TRIM24 and TRIM33 and mechanistic insights have demonstrated a functional interaction between these factors and key regulatory proteins including p53 [71], SMAD transcription factors [72] and oestrogen receptors [73]. Infact, TRIM24 has recently been shown to mediate an aberrant oestrogen response in breast cancer via a specific interaction with modified and unmodified residues on the tail of histone H3 [73].

CHROMATIN REMODELLERS

Modifications of the nucleosome can directly influence the compaction state of chromatin by altering the affinity of the histone-DNA interaction. In addition to this, the active reorganization of nucleosome positioning is often necessary to initiate transcription. In mammals, four major complexes of chromatin remodellers have been identified: the switching defective/sucrose non-fermenting (SWI/SNF) family, the imitation SWI family, the nucleosome remodelling and deacetylation/Mi-2/chromodomain helicase DNA-binding family (NuRD) and the inositol requiring 80 family. These remodelling complexes are capable of both shifting and evicting nucleosomes. The energy obtained from the ATPase activity is used to destabilize the interaction between DNA and histones. The chromatin remodelling activity of the SWI/SNF complex in mammalian cells can induce both transcriptional activation and transcriptional repression. Activation is achieved by the creation of loops of open chromatin that are accessible to transcription factors, whilst repression is achieved by concentrating nucleosomes at specific genomic regions and reducing the accessibility of DNA to the transcriptional machinery.

The SWI/SNF complexes are formed by the physical interaction of 9–12 protein subunits. The core subgroup of proteins that are always present in the complex include SNF5, BAF170, BAF155 and one of the mutually exclusive ATPase subunits SMARCA4 or SMARCA2, both of which contain a BRD. The accessory subunits are often lineage and context specific and tailor the regulation and the targeting of the complexes. In mammalians two distinct subgroups of SWI/SNF complexes can be identified by the presence of specific accessory subunits. The BRG1-associated factor (BAF) complex, also known as SWI/SNF-A, is characterized by the presence of the ARID1A/B subunits; whilst the polybromo-BAF (pBAF) complex, also known as SWI/SNF-B is characterized by the presence of BAF180, BAF200 and BRD7. The BAF complex can associate with both SMARCA4 (BRG1) and SMARCA2 (BRM) catalytic subunits but the pBAF complex is almost exclusively associated with SMARCA4. In mammals, the expression of several components of the SWI/SNF complexes is tissue specific, generating variants of the remodelling complexes that direct cell fate by facilitating lineage-specific gene expression programmes.

Several members of the SWI/SNF complexes are involved in cancer. In particular, the core subunit SNF5 and the ATPase subunits SMARCA2 and SMARCA4 are often downregulated or deleted in variety of cancers suggesting a tumour-suppressor role for these complex members [74].

The expression of BRG1 is markedly reduced in 20–50% of primary human lung cancers [75,76]. However, experimental evidence would suggest that gene dosage and cellular context play a significant role in determining the functional outcome from pertubations in BRG1. BRG1 haploinsufficiency in lung cells was initially demonstrated to accelerate carcinogenesis induced by ethyl carbamate but interestingly homozygous deletion of BRG prevented the formation of lung tumours [77]. Furthermore, if the homozygous deletion of BRG1

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is induced after the treatment with the carcinogen it now promotes carcinogenesis [77]. Further evidence to support the hypothesis that gene dosage is important is seen in a haploinsufficient murine model where one of the *brg1* alleles is deleted. These mice develop mammary tumours that retain the expression of the second *brg1* allele indicating that a residual ATPase activity is necessary for tumour cells [78].

The wealth of genomic and functional information implicating the SWI/SNF complexes in cancer would suggest that aberrant chromatin localization via one of the many BRD containing proteins to facilitate oncogenic gene expression programmes is likely to be involved. Therefore, small molecules that specific perturb the BRD-mediated association with chromatin may find a therapeutic niche in certain malignancies.

CHROMATIN MODIFIERS

Several members of the BRD family are direct chromatin modifiers. These enzymes may use their BRD to bind to chromatin and deposit and spread new modifications on neighbouring nucleosomes facilitating specific biological outcomes.

An example of this group is the cyclic AMP response element-binding (CREB) protein (CBP) and the highly homologous p300, which are histone acetyltransferases and transcriptional cofactors that contain a single BRD [79]. CBP/p300 also function as a scaffold for various members of the transcriptional machinery and/or transcription factors [80,81]. Together, CBP/p300 localize to acetylated chromatin via their BRD, modify chromatin through their HAT activity and recruit the transcriptional machinery to potentiate gene expression. CBP is also able to bind acetylated lysines on non-histone proteins, such as p53, and this interaction is required for the activation of the cyclin-dependent kinase inhibitor p21 [82]. These functions have led to many studies that have drawn associations with CBP/p300 activity and the development, maintenance or progression of cancer. In rare cases of acute myeloid leukaemia CBP, and less frequently p300, are found fused with the monocytic leukaemia zinc-finger protein (MOZ) or MLL [83–85]. When present, these fusions are often the initiating event in the transformation process. These proteins have also been ascribed an oncogenic function in prostate cancer [86]. Here, CBP and p300 function as cofactors of the androgen receptor (AR) and drive the progression of prostate cancer [87]. Interestingly, they are induced by androgen deprivation and can induce androgen-dependent genes in castration-resistant cancers even in the presence of very low AR levels [88]. Further evidence to support their oncogenic potential is derived from the observation that inhibition of the acetyltransferase activity of CBP-p300 induces apoptosis in prostate cancer cells [89]. In contrast to its reported oncogenic function, inactivating mutations of CBP have also been described in a range of haematological and solid malignancies [37,36]. The functional consequences of these inactivating mutations have not been explored in depth but serve to highlight the fact that, like many chromatin regulators, CBP/P300 may act as oncogenes or tumour suppressors depending on the cellular context. As the BRD of CBP/p300 serves to localize their activity at chromatin these could represent an exciting therapeutic target if applied in the correct context and malignancy.

CLINICAL APPLICATION

The exciting possibility of translating these novel epigenetic therapies into the clinical arena has already begun with the BET inhibitors. The results of these early stage clinical trials are eagerly awaited not only from an efficacy point of view but also from a toxicity perspective. Many of the proteins discussed earlier play a fundamental role in cellular homeostasis and as such one would expect that perturbation of their function might have detrimental effects in normal tissues. Interestingly, this prediction has not been a major issue thus far in the pre-clinical characterization of these compounds. Using the BET inhibitors as an example, *in vitro* studies with these compounds on mobilized haematopoietic stem cells show only a modest effect on their clonogenic capacity in comparison with the near complete inhibition observed in the leukaemia initiating cells [17,65]. Similarly, *in vivo* studies with these compounds at doses that have significant anti-tumour activity does not led to widespread systemic toxicity as evidenced by a lack of significant anorexia, diarrhoea or a discernable nadir in blood counts [17,65–67]. Nonetheless, it should be recognized that treatment with these compounds results in a reversible sterility consequent to the inhibition of BRDT [90,91]. Whilst the pre-clinical characterization of these compounds is an imperative step in the process of clinical translation, these studies are limited

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by their scope and duration. The ultimate proof of clinical utility will be gleaned from the ensuing clinical trials in human subjects.

CONCLUDING REMARKS

The last decade has witnessed some of the most impressive advances in human biology. The advent of new technologies and innovative platforms has ushered us into the 'Omic' era, one that promises an unparalleled opportunity to tackle human disease. Whilst the fundamental principle in oncology, that cancer is a disease initiated and driven by genetic anomalies remains uncontested, we are now more than ever aware that this is not the whole story. Many of the hallmarks of cancer, such as malignant self-renewal, differentiation blockade, evasion of cell death and tissue invasiveness are profoundly influenced by changes in the epigenome. Moreover, unlike genomic aberrations, the plasticity of the epigenome provides the perfect milieu for therapeutic intervention. Recognition of these facts has led to a flood of interest in developing novel cancer therapeutics aimed at chromatin regulators. A testament to this progressive approach has been the FDA approval of DNA methyltransferase inhibitors and HDAC-I, which have already made a substantial clinical impact. The pre-clinical promise offered by inhibitors that specifically target the BRDs of BET proteins is a further paradigm shift in our ability to manipulate the epigenome for therapeutic gain. With improved technologies and the increasing interest of the scientific community the stage is now set to make some fundamental inroads into the fight against cancer.

Key Points

- Targeting the epigenome is an emerging therapeutic paradigm in cancer biology.
- BRD containing proteins play a diverse role in regulating various biological processes.
- The normal cellular function of several BRD proteins is perturbed in the initiation and maintenance of various malignancies.
- Advances in structural biology and medicinal chemistry have enabled the development of specific small molecule inhibitors to BRD proteins.

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References

1. Dawson MA, Kouzarides T, Huntly BJ. Targeting epigenetic readers in cancer. *N Engl J Med* 2012;**367**:647–57.
2. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. *Cell* 2012;**150**:12–27.
3. You JS, Jones PA. Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell* 2012;**22**:9–20.
4. Sandoval J, Esteller M. Cancer epigenomics: beyond genomics. *Curr Opin Genet Dev* 2012;**22**:50–5.
5. Shih AH, Abdel-Wahab O, Patel JP, *et al*. The role of mutations in epigenetic regulators in myeloid malignancies. *Nat Rev Cancer* 2012;**12**:599–612.
6. Barneda-Zahonero B, Parra M. Histone deacetylases and cancer. *Mol Oncol* 2012;**6**:579–89.
7. Johnstone R. Histone deacetylase inhibitors in cancer therapyIs transcription the primary target? *Cancer Cell* 2003;**4**:13–8.
8. Prince HM, Dickinson M. Romidepsin for cutaneous T-cell lymphoma. *Clin Cancer Res* 2012;**18**:3509–15.
9. Munster PN, Thurn KT, Thomas S, *et al*. A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. *Br J Cancer* 2011;**104**:1828–35.
10. Yong WP, Goh BC, Soo RA, *et al*. Phase I and pharmacodynamic study of an orally administered novel inhibitor of histone deacetylases, SB939, in patients with refractory solid malignancies. *Ann Oncol* 2011;**22**:2516–22.
11. Huang B, Yang XD, Zhou MM, *et al*. Brd4 coactivates transcriptional activation of NF-kappaB via specific binding to acetylated RelA. *Mol Cell Biol* 2009;**29**:1375–87.
12. Lamonica JM, Deng W, Kadauke S, *et al*. Bromodomain protein Brd3 associates with acetylated GATA1 to promote its chromatin occupancy at erythroid target genes. *Proc Natl Acad Sci U S A* 2011;**108**:E159–168.
13. Mujtaba S, Zeng L, Zhou MM. Structure and acetyl-lysine recognition of the bromodomain. *Oncogene* 2007;**26**:5521–7.
14. Dhalluin C, Carlson JE, Zeng L, *et al*. Structure and ligand of a histone acetyltransferase bromodomain. *Nature* 1999;**399**:491–6.
15. Prinjha RK, Witherington J, Lee K. Place your BETs: the therapeutic potential of bromodomains. *Trends Pharmacol Sci* 2012;**33**:146–53.
16. Filippakopoulos P, Knapp S. The bromodomain interaction module. *FEBS Lett* 2012;**586**:2692–704.
17. Dawson MA, Prinjha RK, Dittmann A, *et al*. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature* 2011;**478**:529–33.
18. Liu J, Lee W, Jiang Z, *et al*. Genome and transcriptome sequencing of lung cancers reveal diverse mutational and splicing events. *Genome Res* 2012.
19. Ciro M, Prosperini E, Quarto M, *et al*. ATAD2 is a novel cofactor for MYC, overexpressed and amplified in aggressive tumors. *Cancer Res* 2009;**69**:8491–8.
20. Hennig EE, Mikula M, Rubel T, *et al*. Comparative kinome analysis to identify putative colon tumor biomarkers. *J Mol Med (Berl)* 2012;**90**:447–56.
21. Zhou Y, Schmitz KM, Mayer C, *et al*. Reversible acetylation of the chromatin remodelling complex NoRC is

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required for non-coding RNA-dependent silencing. *Nat Cell Biol* 2009;**11**:1010–6.
22. Jones MH, Hamana N, Nezu J, *et al*. A novel family of bromodomain genes. *Genomics* 2000;**63**:40–5.
23. Nebral K, Denk D, Attarbaschi A, *et al*. Incidence and diversity of PAX5 fusion genes in childhood acute lymphoblastic leukemia. *Leukemia* 2009;**23**:134–43.
24. LeRoy G, Rickards B, Flint SJ. The double bromodomain proteins Brd2 and Brd3 couple histone acetylation to transcription. *Mol Cell* 2008;**30**:51–60.
25. Lenburg ME, Sinha A, Faller DV, *et al*. Tumor-specific and proliferation-specific gene expression typifies murine transgenic B cell lymphomagenesis. *J Biol Chem* 2007;**282**: 4803–11.
26. French CA, Ramirez CL, Kolmakova J, *et al*. BRD-NUT oncoproteins: a family of closely related nuclear proteins that block epithelial differentiation and maintain the growth of carcinoma cells. *Oncogene* 2008;**27**:2237–42.
27. Thompson-Wicking K, Francis RW, Stirnweiss A, *et al*. Novel BRD4-NUT fusion isoforms increase the pathogenic complexity in NUT midline carcinoma. *Oncogene* 2012.
28. Filippakopoulos P, Qi J, Picaud S, *et al*. Selective inhibition of BET bromodomains. *Nature* 2010;**468**:1067–73.
29. Mantovani F, Drost J, Voorhoeve PM, *et al*. Gene regulation and tumor suppression by the bromodomain-containing protein BRD7. *Cell Cycle* 2010;**9**:2777–81.
30. Yamada HY, Rao CV. BRD8 is a potential chemosensitizing target for spindle poisons in colorectal cancer therapy. *Int J Oncol* 2009;**35**:1101–9.
31. Grunwald C, Koslowski M, Arsiray T, *et al*. Expression of multiple epigenetically regulated cancer/germline genes in nonsmall cell lung cancer. *Int J Cancer* 2006;**118**:2522–8.
32. Laue K, Daujat S, Crump JG, *et al*. The multidomain protein Brpf1 binds histones and is required for Hox gene expression and segmental identity. *Development* 2008;**135**: 1935–46.
33. Kalla C, Nentwich H, Schlotter M, *et al*. Translocation t(X;11)(q13;q23) in B-cell chronic lymphocytic leukemia disrupts two novel genes. *Genes Chromosomes Cancer* 2005; **42**:128–43.
34. Banting GS, Barak O, Ames TM, *et al*. CECR2, a protein involved in neurulation, forms a novel chromatin remodeling complex with SNF2L. *Hum Mol Genet* 2005;**14**:513–24.
35. Chung YR, Schatoff E, Abdel-Wahab O. Epigenetic alterations in hematopoietic malignancies. *Int J Hematol* 2012;**96**: 413–27.
36. Pasqualucci L, Dominguez-Sola D, Chiarenza A, *et al*. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature* 2011;**471**:189–95.
37. Iyer NG, Ozdag H, Caldas C. p300/CBP and cancer. *Oncogene* 2004;**23**:4225–31.
38. Jiang J, Lu J, Lu D, *et al*. Investigation of the acetylation mechanism by GCN5 histone acetyltransferase. *PLoS One* 2012;**7**:e36660.
39. Leachman NT, Brellier F, Ferralli J, *et al*. ATAD2B is a phylogenetically conserved nuclear protein expressed during neuronal differentiation and tumorigenesis. *Dev Growth Differ* 2010;**52**:747–55.
40. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. *Nat Rev Cancer* 2007;**7**:823–33.
41. Varela I, Tarpey P, Raine K, *et al*. Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. *Nature* 2011;**469**:539–42.
42. Farhang-Fallah J, Yin X, Trentin G, *et al*. Cloning and characterization of PHIP, a novel insulin receptor substrate-1 pleckstrin homology domain interacting protein. *J Biol Chem* 2000;**275**:40492–7.
43. Endo M, Yasui K, Zen Y, *et al*. Alterations of the SWI/SNF chromatin remodelling subunit-BRG1 and BRM in hepatocellular carcinoma. *Liver Int* 2012.
44. Schneppenheim R, Fruhwald MC, Gesk S, *et al*. Germline nonsense mutation and somatic inactivation of SMARCA4/ BRG1 in a family with rhabdoid tumor predisposition syndrome. *Am J Hum Genet* 2010;**86**:279–84.
45. Wong AK, Shanahan F, Chen Y, *et al*. BRG1, a component of the SWI-SNF complex, is mutated in multiple human tumor cell lines. *Cancer Res* 2000;**60**:6171–7.
46. Wilson BG, Roberts CWM. SWI/SNF nucleosome remodellers and cancer. *Nat Rev Cancer* 2011;**11**:481–92.
47. Li W, Shang C, Guan C, *et al*. Low expression of Sp100 in laryngeal cancer: correlation with cell differentiation. *Med Sci Monit* 2010;**16**:BR174–178.
48. Pryor JG, Brown-Kipphut BA, Iqbal A, *et al*. Microarray comparative genomic hybridization detection of copy number changes in desmoplastic melanoma and malignant peripheral nerve sheath tumor. *Am J Dermatopathol* 2011;**33**: 780–5.
49. Sille FC, Thomas R, Smith MT, *et al*. Post-GWAS functional characterization of susceptibility variants for chronic lymphocytic leukemia. *PLoS One* 2012;**7**:e29632.
50. Wang PJ, Page DC. Functional substitution for TAF(II)250 by a retroposed homolog that is expressed in human spermatogenesis. *Hum Mol Genet* 2002;**11**:2341–6.
51. Klugbauer S, Rabes HM. The transcription coactivator HTIF1 and a related protein are fused to the RET receptor tyrosine kinase in childhood papillary thyroid carcinomas. *Oncogene* 1999;**18**:4388–93.
52. Friedman JR, Fredericks WJ, Jensen DE, *et al*. KAP-1, a novel corepressor for the highly conserved KRAB repression domain. *Genes Dev* 1996;**10**:2067–78.
53. Khetchoumian K, Teletin M, Mark M, *et al*. TIF1delta, a novel HP1-interacting member of the transcriptional intermediary factor 1 (TIF1) family expressed by elongating spermatids. *J Biol Chem* 2004;**279**:48329–41.
54. Huang H, Rambaldi I, Daniels E, *et al*. Expression of the Wdr9 gene and protein products during mouse development. *Dev Dyn* 2003;**227**:608–14.
55. Bierkens M, Krijgsman O, Wilting SM, *et al*. Focal aberrations indicate EYA2 and hsa-miR-375 as oncogene and tumor suppressor in cervical carcinogenesis. *Genes Chromosomes Cancer* 2012.
56. Masselink H, Bernards R. The adenovirus E1A binding protein BS69 is a corepressor of transcription through recruitment of N-CoR. *Oncogene* 2000;**19**:1538–46.
57. Chung CW, Dean AW, Woolven JM, *et al*. Fragment-based discovery of bromodomain inhibitors part 1: inhibitor binding modes and implications for lead discovery. *J Med Chem* 2012;**55**(2):576–86.
58. Zeng L, Li J, Muller M, *et al*. Selective small molecules blocking HIV-1 Tat and coactivator PCAF association. *J Am Chem Soc* 2005;**127**:2376–7.

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59. Sachchidanand, Resnick-Silverman L, Yan S, *et al*. Target structure-based discovery of small molecules that block human p53 and CREB binding protein association. *Chem Biol* 2006;**13**:81–90.
60. Nicodeme E, Jeffrey KL, Schaefer U, *et al*. Suppression of inflammation by a synthetic histone mimic. *Nature* 2010; **468**:1119–23.
61. French CA, Miyoshi I, Kubonishi I, *et al*. BRD4-NUT fusion oncogene: a novel mechanism in aggressive carcinoma. *Cancer Res* 2003;**63**:304–7.
62. You J, Croyle JL, Nishimura A, *et al*. Interaction of the bovine papillomavirus E2 protein with Brd4 tethers the viral DNA to host mitotic chromosomes. *Cell* 2004;**117**: 349–60.
63. Muntean AG, Tan J, Sitwala K, *et al*. The PAF complex synergizes with MLL fusion proteins at HOX loci to promote leukemogenesis. *Cancer Cell* 2010;**17**: 609–21.
64. Lin C, Smith ER, Takahashi H, *et al*. AFF4, a component of the ELL/P-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia. *Mol Cell* 2010;**37**: 429–37.
65. Zuber J, Shi J, Wang E, *et al*. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature* 2011;**478**:524–8.
66. Delmore JE, Issa GC, Lemieux ME, *et al*. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* 2011;**146**(6):904–17.
67. Mertz JA, Conery AR, Bryant BM, *et al*. Targeting MYC dependence in cancer by inhibiting BET bromodomains. *Proc Natl Acad Sci U S A* 2011;**108**: 16669–74.
68. Belloni E, Trubia M, Gasparini P, *et al*. 8p11 myeloproliferative syndrome with a novel t(7;8) translocation leading to fusion of the FGFR1 and TIF1 genes. *Genes Chromosomes Cancer* 2005;**42**:320–5.
69. Le Douarin B, Zechel C, Garnier JM, *et al*. The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-raf in the oncogenic protein T18. *EMBO J* 1995;**14**:2020–33.
70. Chambon M, Orsetti B, Berthe ML, *et al*. Prognostic significance of TRIM24/TIF-1alpha gene expression in breast cancer. *Am J Pathol* 2011;**178**:1461–9.
71. Allton K, Jain AK, Herz HM, *et al*. Trim24 targets endogenous p53 for degradation. *Proc Natl Acad Sci U S A* 2009; **106**:11612–6.
72. Morsut L, Yan KP, Enzo E, *et al*. Negative control of Smad activity by ectodermin/Tif1gamma patterns the mammalian embryo. *Development* 2010;**137**:2571–8.
73. Tsai WW, Wang Z, Yiu TT, *et al*. TRIM24 links a non-canonical histone signature to breast cancer. *Nature* 2010;**468**:927–32.
74. Wilson BG, Roberts CW. SWI/SNF nucleosome remodellers and cancer. *Nat Rev Cancer* 2011;**11**:481–92.
75. Reisman DN, Sciarrotta J, Wang W, *et al*. Loss of BRG1/BRM in human lung cancer cell lines and primary lung cancers: correlation with poor prognosis. *Cancer Res* 2003; **63**:560–6.
76. Medina PP, Romero OA, Kohno T, *et al*. Frequent BRG1/SMARCA4-inactivating mutations in human lung cancer cell lines. *Hum Mutat* 2008;**29**:617–22.
77. Glaros S, Cirrincione GM, Palanca A, *et al*. Targeted knockout of BRG1 potentiates lung cancer development. *Cancer Res* 2008;**68**:3689–96.
78. Bultman SJ, Herschkowitz JI, Godfrey V, *et al*. Characterization of mammary tumors from Brg1 heterozygous mice. *Oncogene* 2008;**27**:460–8.
79. Bannister AJ, Kouzarides T. The CBP co-activator is a histone acetyltransferase. *Nature* 1996;**384**(6610):641–3.
80. Munshi N, Merika M, Yie J, *et al*. Acetylation of HMG I(Y) by CBP turns off IFN beta expression by disrupting the enhanceosome. *Mol Cell* 1998;**2**:457–67.
81. Merika M, Williams AJ, Chen G, *et al*. Recruitment of CBP/p300 by the IFN beta enhanceosome is required for synergistic activation of transcription. *Mol Cell* 1998;**1**: 277–87.
82. Mujtaba S, He Y, Zeng L, *et al*. Structural mechanism of the bromodomain of the coactivator CBP in p53 transcriptional activation. *Mol Cell* 2004;**13**:251–63.
83. Lai JL, Jouet JP, Bauters F, *et al*. Chronic myelogenous leukemia with translocation (8;22): report of a new case. *Cancer Genet Cytogenet* 1985;**17**:365–6.
84. Borrow J, Stanton VP, Jr, Andresen JM, *et al*. The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. *Nat Genet* 1996;**14**:33–41.
85. Chaffanet M, Gressin L, Preudhomme C, *et al*. MOZ is fused to p300 in an acute monocytic leukemia with t(8;22). *Genes Chromosomes Cancer* 2000;**28**:138–44.
86. Heemers HV, Debes JD, Tindall DJ. The role of the transcriptional coactivator p300 in prostate cancer progression. *Adv Exp Med Biol* 2008;**617**:535–40.
87. Bouchal J, Santer FR, Hoschele PP, *et al*. Transcriptional coactivators p300 and CBP stimulate estrogen receptor-beta signaling and regulate cellular events in prostate cancer. *Prostate* 2011;**71**:431–7.
88. Debes JD, Schmidt LJ, Huang H, *et al*. p300 mediates androgen-independent transactivation of the androgen receptor by interleukin 6. *Cancer Res* 2002;**62**:5632–6.
89. Santer FR, Hoschele PP, Oh SJ, *et al*. Inhibition of the acetyltransferases p300 and CBP reveals a targetable function for p300 in the survival and invasion pathways of prostate cancer cell lines. *Mol Cancer Ther* 2011;**10**: 1644–55.
90. Matzuk MM, McKeown MR, Filippakopoulos P, *et al*. Small-molecule inhibition of BRDT for male contraception. *Cell* 2012;**150**(4):673–84.
91. Gaucher J, Boussouar F, Montellier E, *et al*. Bromodomain-dependent stage-specific male genome programming by Brdt. *EMBO J* 2012;**31**:3809–20.

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