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# Bromodomain Coactivators in Cancer, Obesity, Type 2 Diabetes, and Inflammation

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## Abstract

Double bromodomain proteins bind to acetylated lysines in histones, bringing associated histone modification and nucleosome remodeling activity to chromatin. The ability of bromodomain regulators to alter chromatin status and control gene expression has long been appreciated to be important in the development of certain human cancers. However, bromodomain proteins have now been found also to be critical, non-redundant players in diverse, non-malignant phenotypes, directing transcriptional programs that control adipogenesis, energy metabolism and inflammation. The fact that such different processes are functionally linked by the same molecular machinery suggests a common epigenetic basis to understand and interpret the origins of several important co-morbidities, such as asthma or cancer that occurs in obesity, and complex inflammatory diseases like cardiovascular disease, systemic lupus erythematosus, rheumatoid arthritis and insulin resistance that may be built on a common pro-inflammatory foundation.

## Bromodomain-containing Protein Complexes and the Epigenetic Regulation of Transcription

The manner in which cells interpret their environment is critically determined by chromatin control of gene expression. It is now clear that molecular understanding of the etiology of major diseases must look beyond DNA-based mechanisms of genetic mutation and transcription factor targeting. Mechanism must account for epigenetic factors, including the role of the chromatin regulatory machinery: histone modification, nucleosome remodeling, and promoter DNA methylation. Indeed, the epigenetic mechanisms that regulate gene activity are now a focus of rapidly expanding research effort. Numerous reports have investigated how inappropriate targeting of histone modification or chromatin remodeling activity to promoters destabilizes transcriptional networks. Most of the previous insight that has revealed the nature and consequences of chromatin misregulation has come from investigators in yeast genetics and molecular oncology. However, because the transcriptional and chromatin processes involved are so fundamental in biology, we can expect that epigenetics will inform our understanding of a diverse collection of diseases in addition to cancer: inflammatory conditions like asthma (Kabesch *et al*., 2010) and systemic lupus erythematosus (SLE) (Pan *et al*., 2010); disorders of metabolism like metabolic syndrome, dyslipidemia, and Type 2 diabetes (T2D) (Wang *et al*., 2009; Denis *et al*., 2010); and cardiovascular disease (CVD) (Alkemade *et al*., 2010). More importantly, epigenetic

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mechanisms will be seen to be common to the etiology of apparently unrelated diseases that tend to occur simultaneously or sequentially in the same patient as co-morbidities.

Epigenetic events in human and other mammalian cells are regulated by conserved sets of enzymes. Most of these enzymes are already well known; most catalyze specific post-translational modifications of amino acids in the amino- and carboxyl-terminal tails of nucleosomal histones, thus enabling other key factors to bind to these modified histones. The enzymes include DNA methyltransferases, such as cytosine-5 methyltransferases (Kulis and Esteller, 2010); histone lysine acetyltransferases, such as CREB Binding Protein (CBP)/p300 (Bedford *et al.*, 2010) and Tip60 (Sun *et al.*, 2010); histone lysine deacetylases, such as the sirtuins (Bao and Sack, 2010); histone lysine methyltransferases, such as SUV39H1 (Moss and Wallrath 2007) and ASH1 (Schwartz *et al.*, 2010); and histone serine/threonine kinases, such as the mitogen- and stress-activated kinases (Vermeulen *et al.*, 2009). Apart from acetylation and methylation, important histone modifications include phosphorylation of serine 10 in histone H3 (Vermeulen *et al.*, 2009), ubiquitylation (Higashi *et al.*, 2010), sumoylation (Trenkmann *et al.*, 2010), and the introduction into chromatin of variant histones, such as γH2AX (Srivastava *et al.*, 2009), which is a crucial response to DNA damage. Aberrant modification of histones can have dire consequences, including developmental abnormalities (Schwartz *et al.*, 2010) and cancer (Chi *et al.*, 2010). A number of excellent reviews have addressed the diversity and functions of histone modification (Kouzarides, 2007; Chi *et al.*, 2010; Sebova and Fridrichova, 2010). ATP-dependent nucleosome remodeling complexes, such as the “switch mating type/sucrose non-fermenting” (SWI/SNF) (Eisen *et al.*, 1995; Boyer *et al.*, 2000) and ISWI complexes (Eberharter and Becker, 2004), also play a critical role in the establishment of transcriptionally active or silent chromatin. These complexes have newfound significance for stem cell function (Lessard and Crabtree, 2010). An interesting motif called the bromodomain occurs in numerous proteins involved in these chromatin and transcriptional processes; questions regarding bromodomain structure and functional role have come to prominence recently.

The bromodomain is an evolutionarily conserved, ~110 amino acid motif comprised of four left-handed, antiparallel α-helices. The word is etymologically unrelated to bromine, but to brahma, an important *Drosophila* developmental regulator in which the motif was first reported by Kennison and colleagues (Tamkun *et al.*, 1992). This motif is a common feature in a diverse set of proteins united by their importance in transcription co-activation and chromatin structure (Haynes *et al.*, 1992; Jeanmougin *et al.*, 1997). Zhou and colleagues used nuclear magnetic resonance spectroscopy to solve the first structure of a bromodomain, using p300/CBP Associated Factor (P/CAF), and established that the motif associates with ε-acetyl-lysine residues in nucleosomal histones (Dhalluin *et al.*, 1999), as well as with acetylated p53 (Mujtaba *et al.*, 2004). The bromodomain appears to be the only motif that recognizes protein ε-acetyl-lysine (Zeng and Zhou, 2002), which is achieved through interaction of ε-acetyl-lysine ligand with two peptide loops that connect the bromodomain α-helices. A number of human bromodomain structures have now been solved, most importantly for TBP-Associated Factor (TAF)-1 (Jacobson *et al.*, 2000), formerly called Cell Cycle Gene (CCG)-1 or $TAF_{II}250$, which is an essential component of the basal transcription machinery. Among other functions, TAF1 promotes Mdm2-regulated p53 turnover (Allende-Vega *et al.*, 2007) and controls *cyclin A2* transcription (hereafter “cyclin A”) (Wang *et al.*, 1997). Other solved bromodomain proteins of importance are Brg1, a core, catalytic component of the SWI/SNF chromatin-remodeling complex (Shen *et al.*, 2007), Brd2, (Nakamura *et al.*, 2007) and Brd4 (Liu *et al.*, 2008). In addition to binding to acetylated lysines of nucleosomal histones, bromodomain-containing proteins are often histone acetyltransferases themselves or are associated with histone acetyltransferases, thereby anchoring their transcriptional activator function on promoter chromatin (Yang,

2004). The field has been extensively and effectively reviewed (Taverna *et al.*, 2007; Wu and Chiang, 2007; Denis *et al.*, 2010).

A subset of bromodomain protein complexes exhibits dual character. They recruit either transcriptional co-activators or co-repressors depending on the requirements of the signal transduction machinery and the promoter (Denis, 2001a). For example, variant SWI/SNF chromatin remodeling complexes (with which Brd2 associates; Denis *et al.*, 2006; Romesser *et al.*, 2009) exert opposing effects in cell cycle control (Nagl *et al.*, 2006; 2007). Recent work suggests another mechanism for the switch between activating and repressing chromatin complexes: different tissue-specific forms of bromodomain proteins recruit different epigenetic regulators or transcription factors to chromatin. Specifically, the long form of Brd2, formerly called RING3, ordinarily co-represses peroxisome-proliferator-activated receptor gamma (PPARγ) target genes in adipocytes and the insulin gene in pancreatic β cells. Reduced Brd2 expression derepresses these target genes (Wang *et al.*, 2009). Conversely, the short form of Brd2 ordinarily co-activates cyclin A in proliferating cells, in part through recruitment of histone acetyltransferase activity (Greenwald *et al.*, 2004; Sinha *et al.*, 2005). It follows that Brd2 forms might be functionally analogous to E2F forms (with which Brd2 proteins also associate; Denis *et al.*, 2000), inasmuch as E2F1-3 promote proliferation while E2F4-8 promote differentiation (Wu *et al.*, 2001).

## Single Bromodomain Proteins in Cancer

Improper histone acetylation causes certain hematologic malignancies. In acute promyelocytic leukemia, for example, a histone acetyltransferase replaces the Nuclear Co-Repressor (NCoR)/Sin3/histone deacetylase (HDAC) repression complex, resulting in inappropriate transactivation of genes (Martens *et al.*, 2010). Similarly, exchanged recruitment of coactivator for corepressor is the mechanism by which the oncoprotein AML1-ETO alters gene expression and accounts for >10% of acute myeloid leukemia (AML) (Redner *et al.*, 1999). Epigenetically important proteins that contain a single bromodomain also play critical roles in malignancy. It has been long appreciated, for example, that chromosomal translocations can mistarget histone modification enzymes or chromatin remodeling machines to incorrect promoters, accounting for a significant number of hematologic malignancies (Redner *et al.*, 1999). The t(8;16)(p11;p13) associated with the M4/M5 subtype of AML is the first report of a translocation involving the histone acetyltransferase CBP (Borrow *et al.*, 1996). In another case, the t(11:16)(q23;p13.3), arising in treatment-related myelodysplasias and AML, fuses the mixed lineage leukemia gene (*MLL*) to *CBP* (Sobulo *et al.*, 1997). Full oncogenicity of MLL-CBP is retained only if both the histone acetyltransferase activity and the bromodomain of CBP are present in the transforming fusion gene (Lavau *et al.*, 2000). Fusion of the bromodomain-containing p300 acetyltransferase to monocytic leukemia zinc finger protein (MOZ) in certain acute monocytic leukemias harboring a t(8;22)(p11;q13) chromosome translocation has also been reported (Kitabayashi *et al.*, 2001). Finally, a carboxyl-terminal truncation of p300, which also serves as a co-activator for transactivation of human *c-rel*/protooncogene [REL, a transcription factor in the Nuclear Factor-κB (NF-κB) family] target genes, is expressed in the RC-K8 cell line, which is of a diffuse large B cell lymphoma (DLBCL) origin (Garbati *et al.*, 2010). Thus, a major class of oncogenic mutation is defined by improper histone acetylation and transcriptional co-activation as a consequence of bromodomain protein abnormality (Panagopoulos *et al.*, 2001; Shigeno *et al.*, 2004; Serravalle *et al.*, 2010). This epigenetic analysis of oncogenic mechanism has proven useful to identify transcriptional networks in acute leukemic patients and to classify these patients more accurately for the purposes of risk assessment and treatment decisions (Figueroa *et al.*, 2008).

Other single bromodomain proteins of importance in cancer include Atad2, which is a co-activator of Myc transcription in hormone-responsive human tumors of the breast and prostate (Ciro *et al.*, 2009). High Atad2 levels increase short-term mortality in lung and breast cancer patients (Caron *et al.*, 2010). In addition, Brd7 physically and functionally interacts with well known tumor suppressors such as Brca1 (Harte *et al.*, 2010) and p53 (Drost *et al.*, 2010). *BRD7*, located in humans at 16q12, encodes tumor suppressor functions; the gene is frequently deleted in breast tumors that harbor wild type p53 and is required for p53-dependent replicative senescence (Burrows *et al.*, 2010; Drost *et al.*, 2010).

The role of DNA methylation (Kampranis and Tsichlis, 2009), histone modification (Sebova and Fridrichova, 2010), and related epigenetic mechanisms in carcinogenesis is now widely appreciated, as evidenced by the development of novel histone deacetylase inhibitors such as vorinostat (Cang *et al.*, 2009). Yet the effects of these agents are widely distributed throughout the genome (Mogal and Abdulkadir, 2006) and interact with pattern-forming transcription factors (Chen *et al.*, 2010) in complex networks during normal development and differentiation. Better design of epigenetically-directed cancer therapeutics with minimal side effects will require a more sophisticated understanding of these networks and how chromatin interprets mitogenic signal information to regulate the transcriptional outcome of cell cycle genes.

## Double Bromodomain Proteins in Cancer

Certain members of the double bromodomain protein family, which includes Brd2, Brd3, Brd4, and Brd6, have emerged over the last decade as major epigenetic regulators of proliferation, differentiation, and human cancer. Brd2 and Brd4 appear to play particularly important roles. The human *BRD4* gene, located at 19p13.1, affects breast cancer microenvironment and cancer survival (Crawford *et al.*, 2008). *BRD4* is involved in rare but recurrent, reciprocal chromosomal translocations with the gene “nuclear protein in testis” (*NUT*) at 15q14 that produce a *BRD4-NUT* fusion oncogene. The fusion protein gives rise to a highly lethal, poorly differentiated neoplasm called NUT midline carcinoma (NMC) (French *et al.*, 2001). The *BRD4-NUT* lesion blocks differentiation and promotes proliferation (French, 2010). Small molecules that disrupt bromodomain interactions with chromatin, initially suggested by peptide inhibitor studies (Dey *et al.*, 2003; Mujtaba *et al.*, 2004; Sanchez and Zhou, 2009), have recently been shown to be of possible therapeutic benefit for *BRD4-NUT* tumors (Filippakopoulos *et al.*, 2010). In view of the severe side effect profiles for many traditional antimetabolite and genotoxic therapies for human malignancy, such novel and targeted “epigenetic therapeutics” are welcome. Likewise, small molecules that mimic histones and can compete for the bromodomain binding pocket may prove useful as therapeutics for inflammation, especially if discrimination between Brd2, Brd3, and Brd4-regulated target genes can be improved (Nicodeme *et al.*, 2010).

Brd4 is also important in Kaposi’s sarcoma (KS), where it has quite a different function. KS is caused by a human gamma-2 herpesvirus; during latent infection, herpesvirus genomes are stably maintained as multi-copy circular episomes in the nuclei of infected cells. Transmission of viral genomes to daughter cells during mitosis is achieved through interaction of the episomes with KS-associated herpesvirus-encoded Latency-Associated Nuclear Antigen 1 (LANA), one of the products of the latency genes of the virus. Brd4 provides a chromatin anchor for LANA and viral episomes (You *et al.*, 2006) and thus is important for disease persistence. Brd4 may also play a role in transcriptional networks that are directed by LANA (Verma and Robertson, 2003). Animal and human papillomaviruses (HPV), certain high-risk types of which are the major cause of cervical cancer during persistent HPV infection, can also use Brd4 as a cellular adaptor to anchor viral genomes to mitotic chromosomes (McPhillips *et al.*, 2005), in a similar manner to KS-associated

herpesvirus. Both Brd2 (Nakamura *et al.*, 2007) and Brd4 (Dey *et al.*, 2003) bind acetylated histones and mobilize chromatin modification (Wu and Chiang, 2007) to control cell cycle (Denis *et al.*, 2000; Dey *et al.*, 2000). Brd4 thus plays a fundamental role in cell cycle and transcriptional programs that are important in cancer (Jang *et al.*, 2005, Yang *et al.*, 2005) and viral transformation.

Brd2 bromodomains are highly homologous to those of TAF1, the cell cycle regulator with which Brd2 was first compared (Beck *et al.*, 1992). Brd2, a mitogen-responsive, nuclear-localized protein kinase, is also a homolog of *female sterile homeotic*, which is an activator of *trithorax* in *Drosophila* (Mozer and Dawid, 1989). Brd2 likely co-activates *MLL* target genes in 11q23 mixed lineage leukemias (Guo *et al.*, 2000). Through its bromodomains and carboxyl-terminal domain for association with E2F-containing protein complexes, Brd2 provides a scaffold on chromatin (Denis, 2001b) that recruits histone acetyltransferase and chromatin remodeling activities (Denis *et al.*, 2006) to the cyclin A promoter (Sinha *et al.*, 2005), thereby coupling histone acetylation to transcription (LeRoy *et al.*, 2008). B cell-restricted constitutive expression in mice of Brd2 inappropriately transactivates the cyclin A gene in pre-malignant B cells (Greenwald *et al.*, 2004) to cause a malignancy that is highly similar to human DLBCL (Lenburg *et al.*, 2007). This malignancy exhibits the “activated B cell” (ABC) transcriptional subtype of DLBCL (Greenwald *et al.*, 2004). In humans, ABC DLBCL features constitutive activation of the NF-κB pathway and improved survival, unlike the “germinal center B” (GCB) subtype (Bea *et al.*, 2005). ABC is a more aggressive type of DLBCL than GCB and is associated with poor survival. The relationship between NF-κB family members and inflammatory signal transduction in ABC DLBCL has been intensively investigated. In mice, the Brd2-driven ABC DLBCL can be cured with a standard regimen of Cyclophosphamide, Hydroxydaunorubicin (adriamycin), Oncovin (vincristine), and Prednisone (CHOP) (Longe *et al.*, 2009) that is also used for human ABC DLBCL. This model has been used to resolve a proliferation signature from a malignancy signature for novel target discovery, in both transcriptional (Lenburg *et al.*, 2007) and proteomic terms (Romesser *et al.*, 2009). Despite strong evidence that further understanding of bromodomain function may lead to novel insights into cancer control, only one other mouse model of bromodomain-dependent hematopoietic malignancy has been reported (Liedman and Zeleznik-Le, 2001). New models are vitally needed to explore molecular mechanisms of malignant transformation by Brd2 and Brd4, e.g., transcriptional deregulation in KS-associated herpesvirus (You *et al.*, 2006), and to develop novel bromodomain-directed therapies for the relevant cancers (Filippakopoulos *et al.*, 2010).

## A Major Surprise: Brd2 in Obesity and Type 2 Diabetes

Experiments to delete *BRD2* or *BRD4* genes, or knock down expression with shRNA, in order to obtain deeper, mechanistic information about Brd2 and Brd4 function in normal proliferation, have been difficult because Brd2 and Brd4 are essential for cell growth. The null phenotype of *brd2(−/−), brd4(−/−)* or their homologs is lethal in yeast (Chua and Roeder, 1995), *Drosophila* (Digan *et al.*, 1986; Haynes *et al.*, 1989), and mice (Houzelstein *et al.*, 2002; Gyuris *et al.*, 2009; Shang *et al.*, 2009). However, insight was achieved recently with the accidental discovery of a Brd2 hypomorphic phenotype in mice engineered from *Brd2* gene-disrupted embryonic stem cells (Wang *et al.*, 2009). These mice on the C57BL6/J background harbor a *lacZ* gene insertion in the 5′ controlling regions of the endogenous *Brd2* gene. Rather than causing embryonic lethality, this mutation reduces but does not eliminate Brd2 expression throughout the animal, enabling survival. Heterozygous mice become extremely obese on regular chow diet, whilst avoiding the insulin resistance (IR) that would normally occur on the C57BL6/J background. This observation made clear that double bromodomain proteins, although important for carcinogenesis, are critical and non-redundant in surprisingly diverse processes in the organism, including pancreatic β cell

function, metabolic health, and adipogenesis (Wang *et al.*, 2009). The functions of double bromodomain proteins are likely to have major implications for the etiology of metabolic disease, and in other scenarios where morbidity results from inflammation, as described below.

## Bromodomain Proteins in Inflammation

Insulin resistance (IR) in the context of obesity is associated with a chronic state of inflammation of white adipose tissue and systemic, subclinical inflammation (Bastard *et al.*, 2006; Shoelson *et al.*, 2006), characterized by elevated serum concentrations of C-reactive protein (CRP) (Kahn *et al.*, 2006), interleukin-6 (IL-6), IL-8, monocyte chemotactic protein-1 (MCP-1), and tumor necrosis factor-α (TNF-α) in patients and in different animal models of obesity (Hotamisligil *et al.*, 1993; Kim *et al.*, 2006). TNF-α in particular is broadly important for many acute and chronic inflammatory conditions. Interestingly, 20–30% of the adult obese population remains relatively "metabolically healthy" despite obesity (MHO) (Ruderman *et al.*, 1981*);* they display an absence of impaired glucose tolerance, dyslipidemia, hyperuricemia, and hypertension (Bonora *et al.*, 1991; Wildman *et al.*, 2008). Their metabolic and CVD risk factors are relatively low (Ruderman *et al.*, 1981; Succurro *et al.*, 2008). Protection from common complications of obesity in these MHO patients is attributable in part to a reduced inflammatory profile compared to at-risk obese patients, including less severe elevation of serum CRP and $\alpha_1$-antitrypsin (A1AT) (Karelis *et al.*, 2005). MHO patients show less severe elevation of TGF-β1, plasminogen activator inhibitor-1, activated factor VII, and prothrombin fragment 1 + 2 (Romano *et al.*, 2003), and have significantly higher total and high molecular weight adiponectin (Elisha *et al.*, 2010), which is protective of insulin sensitivity. The MHO phenotype may also feature some uncoupling of inflammatory signal transduction from obesity-driven IR (Wang *et al.*, 2009; Belkina *et al.*, 2010), but this hypothesis has not been tested explicitly in humans. A large body of research has identified chronic inflammation as a common factor in diverse diseases, including CVD (Pieringer and Pichler, 2010), IR and metabolic syndrome (Bastard *et al.*, 2006; Shoelson *et al.*, 2006; Suganami and Ogawa, 2010), allergy and asthma (Broide *et al.*, 1992), inflammatory bowel diseases (Crohn's disease and ulcerative colitis; Shen and Durum, 2010), rheumatoid arthritis (RA) (Westlake *et al.*, 2010), SLE (Nalbandian *et al.*, 2009), and cancer (Coussens and Werb, 2002). Transcriptional control of the genes that regulate inflammatory responses is a central mechanism in the etiology of these diseases. Therefore it is reasonable to hypothesize that the "hypo-inflammatory" MHO phenotype may in some individuals reach beyond IR and CVD to protect against the inflammatory components of other diseases, such as asthma and cancer.

The carcinogenic consequences of chronic inflammation are beyond dispute. Very early in the development of molecular oncology as a field, mucosal inflammation was recognized as a primary path leading from tissue injury or occupational exposure to malignancy (Dunham, 1972). Mucosal cancers are easily grasped as sequelae to DNA damage arising from environmental exposure to mutagens and irritants. A full treatment of the role of inflammation in cancer is well beyond the scope of the current review. However, it is pertinent that serious attention is now being paid to the role of obesity and obesity-associated inflammation in cancers (Hursting and Berger, 2010). Signaling through leptin, which is elevated in obesity, engages in cross-talk with STAT3/Jak2-dependent cytokine signaling and AMP-activated protein kinase (Lim *et al.*, 2010). This pathway identifies a potential mechanism to explain the increased incidence of certain cancers, such as colorectal cancer, which is associated with elevated serum leptin (Stattin *et al.*, 2004). Reduced levels of the protective factor adiponectin, such as those present in the serum of obese, insulin resistant patients, are also associated with higher incidence of colorectal (Wei *et al.*, 2005) and breast cancers (Tian *et al.*, 2007). Weight gain among survivors of diverse types of

cancers is common and is associated with poor prognosis (Thomson and Thompson, 2009). Finally, populations at high risk for obesity exhibit greater morbidity rates from breast cancer (Vona-Davis and Rose, 2009) and other cancers for which obesity is a risk factor. However, the links between bromodomain-regulated target genes, inflammation, and carcinogenesis are only just beginning to be studied.

Much research effort is currently being expended to identify the genetic loci and functional single nucleotide polymorphisms (SNPs) that mediate human susceptibility to the inflammation-driven diseases and co-morbidities. These tools would enable better monitoring of at-risk individuals and biomarker-based prescription of aggressive treatment protocols. However, such biomarkers are of more use when they illuminate mechanism. The highly attenuated inflammation in the white adipose tissue of Brd2 hypomorphic mice that develop severe obesity on a regular chow diet (Wang *et al.*, 2009) suggests a protective mechanism that might be relevant to the MHO population. Brd2-deficient bone marrow-derived macrophages dramatically under-produce several pro-inflammatory cytokines when challenged with bacterial endotoxin (Belkina *et al.*, 2010). In addition, recent data show that synthetic histone mimetics can disrupt the binding of double bromodomain proteins like Brd2 to histones, and thereby ablate the transcription of a broad spectrum of inflammatory cytokine genes in murine bone marrow-derived macrophages challenged with bacterial endotoxin, including genes that encode IL-1α, IL-1β, IL-6, IL-12α, IL-23α, IL-27, and serum amyloid A3; the chemokine factors CCL5, CCR5, CXCL3, CXCL9, and CXCL10; and the activation markers CD69 and CD86; all of which are NF-κB responsive — although interestingly, not TNF-α — and reduce inflammation *in vivo* (Nicodeme *et al.*, 2010). Brd2-directed siRNA also knocks down IL-1β and IL-6 transcription in this model. These new data strongly support the hypotheses that (1) Brd2 is a critical regulator of inflammation in both adipocytes (Wang *et al.*, 2009) and adipose tissue-infiltrating inflammatory macrophages (Belkina *et al.*, 2010), and that (2) low levels of Brd2 might identify a biomarker and might in part account for the mechanisms that protect MHO individuals from the inflammatory complications of their obesity, including T2D and CVD (Belkina and Denis, 2010).

In humans, the *BRD2* gene resides on Chromosome 6p at 21.3 within the class II major histocompatibility complex (MHC) and the syntenic region of Chromosome 17 in the mouse genome. The 6p21.3 region is highly polymorphic and densely packed with human leukocyte antigen (HLA)-associated genes that are important for diverse inflammatory conditions (de Bakker *et al.*, 2006). This functional link was first appreciated for inflammatory bowel disease (Gleeson *et al.*, 1972), and later broadened to include RA (Gregersen *et al.*, 1987) and immune diseases that involve the class II loci *HLA-DRB1* and *HLA-DQB1*, particularly Type 1 diabetes (T1D) (Todd *et al.*, 1987; Cucca *et al.*, 2001), SLE (Graham *et al.*, 2002), and asthma (Li *et al.*, 2010). Intriguingly, several genes in the 6p21.3 region also encode histones. The 6p21.3 region also includes the *TNF* superfamily cluster, which is located in the class III region, and harbors the genes that encode TNF-α and the closely related factor lymphotoxin (LT)-α, which plays a significant role in the inflammatory component of coronary artery disease (Ozaki *et al.*, 2002). *TNF* is also thought to contribute to the intensity of airway hyperresponsiveness in asthma in the context of obesity (Johnston *et al.*, 2007). The MHC class II cluster of genes at 6p21 is associated with 755 cases of chromosomal abnormality that occur in hematologic and other cancers (Mitelman *et al.*, 2010), yet, reports of polymorphisms that directly link *BRD2* to human cancer, inflammation, or obesity have been lacking. The highly polymorphic nature and linkage disequilibrium of the 6p21.3 region has slowed progress on the fine-structure mapping of loci important for diverse diseases and in particular for co-morbidities. Nevertheless, *BRD2* polymorphism has recently been linked to RA through statistically significant association of three SNPs (Mahdi *et al.*, 2009). As mapping improves, it is likely

that *BRD2* will be implicated in additional co-morbidities of inflammation, cancer, and obesity.

## Bromodomain Proteins in Development

There is strong evidence that Brd2 and its homologs in *Drosophila* (Digan *et al.*, 1986; Haynes *et al*., 1989; Chang *et al.*, 2007), zebrafish (Dibenedetto *et al.*, 2008), and mice (Gyuris *et al.*, 2009; Shang *et al.*, 2009) play crucial, fundamental roles in development and cell fate. Tissue specific conditional and inducible mutants will be necessary to address this question in the case of both Brd2 and Brd4 because of the early lethality seen in null mice (Dey *et al.*, 2000; Houzelstein *et al.*, 2002). Interestingly, Trowsdale and colleagues have noted that *BRD2* has a double bromodomain-encoding paralog at 9q34, called *BRD3* (formerly *ORFX*) (Thorpe *et al.*, 1997), but the functional role of Brd3 remains unknown. Analysis of *BRD3* gene structure and the nearby homologs such as *RXRB*, which encodes retinoid X receptor-β, *PBX2*, which encodes pre-B cell leukemia homeobox 2, and *NOTCH4* suggests this region of 9q34 is part of an ancient duplication of the MHC (Kasahara *et al.*, 1996). Both *BRD2* and *BRD3* are flanked by putative binding sites for NF-κB and the *Drosophila* transcription factors bicoid and Krüppel, which contribute to pattern formation during development (Thorpe *et al.*, 1997). Thus, it is reasonable to predict that *BRD2* polymorphisms or aberrant signaling in the Brd2 pathway, or other mutations in the double bromodomain family genes, will be found to play a role in human development, organogenesis, or metabolic “set-point.”

## Conclusion

Translocations of the double bromodomain co-regulator Brd4 can be oncogenic in humans; constitutive expression of the closely related protein Brd2 is oncogenic in mouse models. New data support the novel hypothesis that abnormalities in signaling through Brd2 or other double bromodomain proteins underlies human predisposition to elevated body mass index and altered insulin sensitivity in adults. These data also support a link between Brd2 function and predisposition to dyslipidemia or improper regulation of adipogenesis, elevated inflammatory profile and risk for CVD and T2D, and increased susceptibility to autoimmune diseases like RA and SLE. Most importantly, the shared epigenetic machinery may contribute to a combination of these conditions and co-morbidities. We are witnessing an explosion of interest in the epigenetic mechanisms that connect chronic inflammation and complex co-morbidities in patients (Mangge *et al.*, 2010; Ozgen *et al.*, 2010; Westlake *et al.*, 2010). New research will benefit from taking this integrative approach.

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## Abbreviations

| | |
|---|---|
| **ABC** | activated B cell |
| **AML** | acute myeloid leukemia |
| **CBP** | CREB binding protein |

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| **CHOP** | cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone |
| **CRP** | C-reactive protein |
| **CVD** | cardiovascular disease |
| **DLBCL** | diffuse large B cell lymphoma |
| **GCB** | germinal center B cell |
| **HDAC** | histone deacetylase |
| **HLA** | human leukocyte antigen |
| **HPV** | human papillomavirus |
| **IL** | interleukin |
| **IR** | insulin resistance |
| **KS** | Kaposi's sarcoma |
| **LANA** | latency associated nuclear antigen |
| **LT-α** | lymphotoxin-α |
| **MCP** | monocyte chemotactic protein |
| **MHC** | major histocompatibility complex |
| **MHO** | metabolically healthy but obese |
| **MLL** | mixed lineage leukemia |
| **MOZ** | monocytic leukemia zinc finger protein |
| **NCoR** | nuclear co-repressor |
| **NF-κB** | nuclear factor kappa B |
| **NMC** | NUT midline carcinoma |
| **NUT** | nuclear protein in testis |
| **P/CAF** | p300/CBP associated factor |
| **PPAR-γ** | peroxisome-proliferator-activated receptor-γ |
| **RA** | rheumatoid arthritis |
| **SLE** | systemic lupus erythematosus |
| **SNP** | single nucleotide polymorphism |
| **SWI/SNF** | switch mating type/sucrose non-fermenting |
| **T1D** | type 1 diabetes |
| **T2D** | type 2 diabetes |
| **TAF** | TATA-box-binding protein (TBP)-associated factor |
| **TNF-α** | tumor necrosis factor-α |

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