Bromodomain and Extra-Terminal Domain Protein 2 in Multiple Human Diseases

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Running title: BRD2 as a crucial epigenetic regulator
Abstract: Bromodomain and extra-terminal domain protein 2 (BRD2), a member of the Bromodomain and extra-terminal domain (BET) protein family, is a crucial epigenetic regulator with significant function in various diseases and cellular processes. The central function of BRD2 is modulating gene transcription by binding to acetylated lysine residues on histones and transcription factors. This review highlights key findings on BRD2 in recent years, emphasizing its roles in maintaining genomic stability, influencing chromatin spatial organization, and participating in transcriptional regulation. BRD2's diverse functions are underscored by its involvement in diseases such as malignant tumor, neurological disorders, inflammatory conditions, metabolic diseases, and virus infection. Notably, the potential role of BRD2 as a diagnostic marker and therapeutic target is discussed in the context of various diseases. While pan-inhibitors targeting the BET family have shown promise in preclinical studies, a critical need exists for the development of highly selective BRD2 inhibitors. In conclusion, this review offers insights into the multifaceted nature of BRD2 and calls for continued research to unravel its intricate mechanisms and harness its therapeutic potential.

Keywords: BET; BRD2; Bromodomain; BET inhibitor

Significance statement: BRD2 is involved in the occurrence and development of diseases through maintaining genomic stability, influencing chromatin spatial organization, and participating in transcriptional regulation. Targeting BRD2 through Protein Degradation Targeting Complexes (PROTAC) technology is emerging as a promising therapeutic approach for malignant cancer and inflammatory diseases.
1. Introduction

In this review, we provide an overview of the structural and physiological characteristics of BRD2. Furthermore, we examine its function in the contexts of tumorigenesis, neurological disorders, inflammatory conditions, metabolic diseases, and virus infection. Lastly, we provide a brief update on the recent advancements in BET protein inhibitors. These new insights may help people better understand the biological functions of BRD2 and provide new ideas for designing treatment strategies for this target in the future.

1.1 Epigenetics

Epigenetics entails the introduction of heritable chemical modifications to DNA and histones. This mechanism effectively governs gene transcription within the genome without modifying the DNA sequence, consequently precipitating phenotypic alterations (Zhang and Cao, 2019). There are various forms of epigenetic mechanisms, such as DNA modifications (methylation and oxidation), histone post-translational modifications (acetylation, methylation, phosphorylation, ubiquitination, and summation), nucleosome positioning, chromatin remodeling, and changes of non-coding RNA expression levels (Topper, Vaz et al., 2020).

The acetylation of histones is a common post-translational modification, which is mediated by histone acetyltransferase (HAT) and histone deacetylase (HDAC) to reversibly acetylate histones. Acetyl binding proteins can recognize acetylated histones and affect the transcription of target genes by recruiting a series of transcription factors or co-stimulatory/inhibitory factors (Peserico and Simone, 2011). Mutations or abnormal expression of acetyl binding proteins can affect the expression of target genes, disrupt normal cellular life activities, and ultimately cause diseases.

1.2 Bromodomain

The bromodomain (BD) represents an evolutionarily conserved protein-protein interaction domain comprised of approximately 110 amino acid residues. It is adept at discerning acetylated lysine residues on histones and transcription factors, thereby facilitating the recruitment of transcription factors or co-stimulators to target gene loci. This process subsequently triggers the phosphorylation of RNA polymerase II,
culminating in the transcriptional activation of target genes (Sanchez. and Zhou., 2009; Wang, Xu et al., 2022). At present, 46 proteins containing BD domains have been identified in the human genome. Based on their structural characteristics, they can be classified into 8 families, which are named by Roman numbers (I–VIII), respectively (Filippakopoulos, Picaud et al., 2012). The II family, which is also named BET family, includes four members in the human genome, named BRD2 (also known as FSRG1, RING3, RNF3, FSH or D6S113E), BRD3 (also known as ORFX or RING3L), BRD4 (also known as MCAP or HUNK1), and BRDT (also known as BRD6, CT9, or SPGF21). In mice, these genes are named Brd2 (Frg1, Fsrsg1, Nat, Ring3 or Rnf3), Brd3 (Fsrsg2, Orfx or Ringl3), Brd4 (Fsrsg4 or Mcap/Hunk1), and Brdt (Fsrsg3 or Brd6).

Under physiological conditions, except for BRDT, which is specifically expressed in the testes, BRD2/3/4 are widely expressed in mammalian nuclei and play important regulatory roles in cell cycle and differentiation (Sansam, Pietrzak et al., 2018; Lee, Park et al., 2019; Paradise, Galvan et al., 2020; Trivedi, Mehrotra et al., 2020; Caputo, Trasanidis et al., 2021; Tsume-Kajioka, Kimura-Yoshida et al., 2022).

2. Classification and structure of BET protein family

Structurally, a defining characteristic of BET family proteins is the presence of two tandem BD domains at their N-terminus (Figure 1). The BD domain comprises four α-helical segments, with every two α-helices separated by variable ring regions. This configuration creates a hydrophobic cavity capable of recognizing acetylated lysine residues (Fujisawa and Filippakopoulos, 2017). The BD1 and BD2 domains from the same protein have approximately 40% similarity in amino acid residues, indicating their functional similarity (Sheppard, Wang et al., 2020). However, although the structures of the two tandem BD1 and BD2 domains are similar, they play different biological roles through different affinities to target proteins (Patel, Solomon et al., 2021). For example, the acetylation of lysine at positions 7 and 11 of histone H2A Z (K7acK11ac) can be preferentially recognized and bound by the BD2 domain of BET protein, and BRDT preferentially interacts with nucleosomes through BD1 instead of BD2 (Miller, Simon et al., 2016; Patel, Solomon et al., 2021).
Although the BD domain plays an important biological role, the specificity of different proteins within the BET protein family is mainly determined by the C-terminal structure of the protein (Werner, Wang et al., 2020). Both BRD4 and BRDT proteins feature a C-terminal motif (CTD) domain at their C-termini, facilitating their interaction with cyclin T1 and cyclin-dependent kinases 9 (CDK9) within the positive transcription elongation factor b (P-TEFb) complex. CTD interacts with P-TEFb, prevents the binding of P-TEFb to the ribonucleoprotein complex 7SK/HEXIM, and maintains P-TEFb in an inactive state, thereby activating RNA polymerase II to initiate transcription (Jang, Mochizuki et al., 2005; Bisgrove., Mahmoudi. et al., 2007). BRD2 and BRD3 lack the CTD domain, but both have an external domain (ET) domain at the C-terminus of their proteins, which can bind to various chromatin remodeling enzymes and related proteins (NSD3, JMJD6, CHD4, GLTSCR1, ATAD5, etc.) to promote transcriptional activation of target genes (Rahman, Sowa et al., 2011; Wai, Szyszka et al., 2018). In addition, the ET domain of the BET protein can bind to viral proteins, promoting the integration of the viral genome and host DNA, thereby mediating viral infection and replication (Gallay, Blot et al., 2019; Duan, Han et al., 2020; Aiyer, Swapna et al., 2021; Zhou, Han et al., 2021).

While specific inhibitors for individual BET proteins have not been identified, various pan inhibitors targeting the BET protein family have been discovered and have entered the clinical trial stage (Wang, Li et al., 2017). JQ1 is a classic pan inhibitor of BET protein family, which has been found to play an anti-tumor effect in breast cancer, renal cell carcinoma, lymphoma, pancreatic cancer and other tumors. JQ1 can induce apoptosis, inhibit cell cycle and impede DNA repair (Kamijo, Sugaya et al., 2017; Miller, Fehling et al., 2019; Park, Yang et al., 2019; Tan, Wang et al., 2020). However, the acquired resistance and non-targeting nature of pan inhibitors of BET protein family have brought difficulties to its clinical application. For example, phase I study of BAY 1238097 was prematurely terminated because of the occurrence of dose-limiting toxicity at a dose below targeted drug exposure (Postel-Vinay, Herbschleb et al., 2019). Another phase I study reported that only 6.2% patients with
relapsed/refractory lymphoma had an objective response treated with CPI-0610 (Blum, Supko et al., 2022). In addition, typical small molecules inhibited the activity of specific proteins through noncovalently binding (and thus reversibly). Therefore, high drug concentrations must be maintained to ensure active-site occupancy and to sustain the clinical benefit (Salami and Crews, 2017). In recent years, researchers have focused on the targeted inhibitors against the BET protein family.

A new generation of BET protein inhibitors, based on PROTAC technology, can catalyze the ubiquitination of BET target proteins via E3 ubiquitin ligases, leading to the proteasomal degradation of BET proteins. The PROTAC technology requires brief interaction between a small molecule and its target protein to trigger the loss of protein function. Therefore, the PROTAC technology overcomes the undruggable target and poor selectivity of small molecules. Moreover, as the earliest target of PROTAC application, BET protein degraders showed significantly stronger anti-proliferative activity than JQ1, indicating better anti-tumor activity of PROTAC technology compared with typical small molecules (Qin, Hu et al., 2018). ARV-825, MZ1, and dBET series compounds are BET protein inhibitors based on PROTAC technology, which are superior to traditional BET protein family inhibitors in selectivity, anti-tumor ability, and drug resistance to targets (Table 1) (Winter, Buckley et al., 2015; Abruzzese, Bilotta et al., 2016; Raina, Lu et al., 2016; Bai, Zhou et al., 2017; Saenz, Fiskus et al., 2017; Sun, Fiskus et al., 2017; Winter, Mayer et al., 2017; Qin, Hu et al., 2018; Saenz, Fiskus et al., 2018; Xu, Chen et al., 2018; Zhou, Hu et al., 2018; Chen, Xu et al., 2019; Hines, Lartigue et al., 2019; Kregel, Malik et al., 2019; Lim, Damnernsawad et al., 2019; Noblejas-López, Nieto-Jimenez et al., 2019; Otto, Schmidt et al., 2019; Piya, Mu et al., 2019; Rathod, Fu et al., 2019; Shi, Zhang et al., 2019; Cimas, Niza et al., 2020; He, Chen et al., 2020; Jiang, Wei et al., 2020; Jiang, Jiang et al., 2020; Qin, Jin et al., 2020; Saraswat, Patki et al., 2020; Szczepanski, Zhao et al., 2020; Zong, Gu et al., 2020; Bauer, Berghoff et al., 2021; Bond, Craigon et al., 2021; Imaide, Riching et al., 2021; Liao, Qian et al., 2021; Liu, Chen et al., 2021; Noblejas-López, Nieto-Jiménez et al., 2021; Pietrobono, Gaudio et al., 2021; Wang, Kutschat et al., 2021; Wu, Jiang et al., 2021; Deng, Yu et al., 2022;
He, Zan et al., 2022; Liu, Qian et al., 2022; Peter, Eisenwort et al., 2022; Piya, Yang et al., 2022; Wang, Xu et al., 2022; Yang, Hu et al., 2022; Zhang, Peng et al., 2022; Zhang, Gao et al., 2022; He, Ju et al., 2023; Huang, Yao et al., 2023; Ivanov, Milosevic Feenstra et al., 2023; Kim, Choi et al., 2023; Liu, Chen et al., 2023; Rose, Fleming et al., 2023; Wang, Li et al., 2023). In addition, BET inhibitors have also been used in combination with AKT inhibitors, PARP inhibitors, and BCL-2 inhibitors, demonstrating impressive therapeutic effects on tumors (Bevill, Olivares-Quintero et al., 2019; Tian, Chen et al., 2019; Fehling, Miller et al., 2020; Lee, Kang et al., 2020; Shigeta, Lui et al., 2021; Zhang, Cai et al., 2021). Despite the structural similarities among BRD2/3/4, they are not merely redundant in terms of their biological functions, but participate in the transcription process of target genes with different recruitment kinetics, interdependence, and sensitivity to BET inhibitors (Kim, Liu, et al., 2021). The currently developed inhibitors exhibit selectivity towards the BD1 and BD2 domains of the BET protein family, but they still lack specificity for individual members within it. Although preferential degradation of BET degraders to different BET family members have been reported, there is still a lack of targeted degraders of BRD2(Wang, Kutschat et al., 2021). Therefore, future research for BET protein inhibitors will still be urgent to target different members of the BET protein family.

However, PROTAC technology also face the challenge of drug resistance that impair the efficiency of other cancer therapies. The different active of E3 ligases in various tumor types may account for the drug resistance to PROTAC technology (Figure 2)(Békés, Langley et al., 2022). For example, the decreased expression of several E3 ligase targets (such as CRBN, VHL and CUL2) have been shown to involve the resistance to PROTAC technology in cultured cells (Ottis, Palladino et al., 2019; Zhang, Riley-Gillis et al., 2019; Shirasaki, Matthews et al., 2021). Therefore, the different E3 ligases active in malignant tumor cells may be used to promote the efficacy of PROTAC technology. Meanwhile, malignant tumor cells acquire the resistance to PROTAC by the drug efflux pump MDR1 (Kurimchak,
Herrera-Montávez et al., 2022), thus blocking the drug efflux pump may promote the efficiency of PROTAC technology.

3. Biological functions of BRD2

3.1 Participation in transcriptional regulation

One of the most well-established functions of BRD2 is its involvement in the transcriptional regulation of target genes, where it serves a scaffolding-like role (Figure 3). As a 'reader' of lysine acetylation modifications, BRD2 utilizes the BD domain located at its N-terminus to recognize and bind acetylated lysine residues on histones. This action subsequently modulates chromatin accessibility and facilitates the recruitment of transcription factors or co-stimulators, ultimately influencing the transcription of target genes (Semer, Bidon et al., 2019). For example, BRD2 can promote the expression of androgen receptor related genes by interacting with histone H2A. Z (Draker, Ng et al., 2012). Recently, it has been reported that the BD domain of BRD2 preferentially binds to acetylated lysine residues, with the most common recognition motif being AcK-X-X-AcK. For example, the BD domain of BRD2 can simultaneously recognize the K4acK7ac and K7acK11ac motifs of histone H2A. Z, where BD2 has a stronger affinity for K7acK11ac (Patel, Solomon et al., 2021). Furthermore, in addition to the aforementioned recognition of acetylated lysine residues and histone binding, BRD2 can also engage with histones indirectly, independent of acetylated lysine residues. This interaction results in the recruitment of BRD4 and lysine acetyltransferase 7 (KAT7), facilitating transcriptional activation (Izumikawa, Ishikawa et al., 2019).

In addition to interacting with histones, BRD2 can also recognize acetylated lysine residues on transcription factor structures, but not all acetylated motifs can bind to BRD2. For example, the acetylated motifs in the E2F1 and MyoD1 structures of transcription factors can bind to the BD of BRD2, but BRD2 cannot recognize the acetylated motifs on the RelA structure of transcription factors. This interaction relies on diacetylation on the transcription factor motifs, while preferentially binding to BD1 (Patel, Solomon et al., 2021). In addition, BD1 and BD2 of BRD2 can also recognize acetylated lysine residues of histones and transcription factors
simultaneously. For example, while BD2 of BRD2 binds to acetylated lysine residues of histones, BD1 can bind to acetylated lysine residues on RUNX family proteins to form complexes, playing a regulatory role in cell cycle progression (Lee, Park et al., 2019). While our understanding of histone post-translational modifications is relatively extensive, our knowledge regarding the interaction between transcription factors and BRD2 remains limited. This interaction may involve complex and dynamic functional regulatory networks.

BRD2 also plays a pivotal role in phase separation. There are numerous disordered regions on BRD2, which enable it to form a structural basis for liquid-liquid phase separation through weak multivalent interactions between proteins. BRD4 can form phase separated aggregates and aggregate in the target gene super promoter region, which is the key to its powerful regulatory function (Wang, Lu et al., 2022). BRD3 can also form phase separation concentrates with lncRNA DIGIT, which synergistically regulate transcription factors that initiate endodermal differentiation at H3K18ac (Daneshvar, Ardehali et al., 2020). However, although it has been found that BRD2 can form phase separation, its specific biological role is still unknown. As research advances, we may acquire a deeper understanding of BRD2’s role in diseases. The modulation of BRD2-mediated phase separation could potentially emerge as a novel approach for disease treatment.

Despite the pivotal role of BRD2 in gene transcription regulation, its underlying mechanisms remain incompletely elucidated. It is noteworthy that, beyond its involvement in epigenetics, recent studies have revealed that BRD2 can interact with other proteins independently of chromatin binding, implying that BRD2 can impact various cellular processes through diverse mechanisms. For example, BRD2 can form complexes with RUNX3 to affect cell cycle progression, or bind with CTCF to form transcription boundaries. BRD4, which is of the same family as BRD2, has been reported to have kinase and lysine acetyltransferase (KAT) activity, but it is still unknown whether BRD2 has similar functions (Devaiah, Lewis et al., 2012; Devaiah, Case-Borden et al., 2016).

3.2 Participation in the formation of chromatin spatial structure
To compress chromatin containing massive genetic information into the nucleus, eukaryotic chromatin has evolved into extremely complicated spatial structures, presenting different characteristics at different scales. At the megabase scale, it can be divided into open A-region chromatin (transcriptionally active) and closed B-region chromatin (transcriptionally inactive) based on whether the chromatin structure is loose. Chromatin belonging to the same region can encounter each other, while chromatin belonging to different regions tends to repel each other. For a long time, the academic community has lacked a clear understanding of the formation mechanism of chromatin compartmentalization. Recently, a research team has discovered that the BRD2 protein can promote the separation and spatial mixing of open chromatin by integrating Assay for Targeting Accessible Chromatin (ATAC) technology with nanoscale microscopy (Xie, Dong et al., 2022). At a more microscopic scale (ranging from thousands to 100,000 base levels), chromatin structural proteins CTCF and cohesin can form chromatin loops through a 'loop extrusion' model, where CTCF can act as a chromatin barrier to prevent the spread of heterochromatin and act as an "insulator". BRD2 can also co-locate with CTCF. If the CTCF/BRD2 occupying element is disrupted, the transcription program can be extended from one gene to another, indicating that BRD2/CTCF together constitute the boundary of transcription (Hsu, Gilgenast et al., 2017).

3.3 Maintenance of genomic stability

Genomic stability is intricately linked to the health and lifespan of cells. Although the preservation of genetic information is a fundamental cellular process, DNA undergoes various physical and chemical changes in response to various exogenous or endogenous stimuli, collectively known as DNA damage. If DNA is damaged and not repaired in a timely and effective manner, it may cause malignant tumors and neurodegenerative diseases, and may also cause cell and body aging (Olivieri, Cho et al., 2020). BET protein can bind and inhibit ATAD5 complex through its ET domain, thereby regulating the quantity of proliferating cell nuclear antigen (PCNA) on chromatin (Wessel, Mohni et al., 2019). As a negative regulatory factor for transcription related RNA-DNA hybrid chains, BRD2 can directly bind and
activate topoisomerase I, thereby inhibiting the formation of R-loops and avoiding DNA double strand breaks (Kim, Lee et al., 2019). Conversely, the use of the BET inhibitor JQ1 can exacerbate DNA damage in cells (Miller, Fehling et al., 2019). In eukaryotic cells, telomeres serve as a small segment of DNA-protein complex to maintain chromatin integrity, and their damage can cause cell aging and even disease occurrence. In mammals, telomere protection is mediated by the key protein TRF2, which can bind to the chromatin end to protect DNA integrity (Ruis, Van Ly et al., 2021). In recent years, it has been found that the telomeric protective effect of TRF2 also relies on BRD2 (Markiewicz-Potoczny, Lobanova et al., 2021), indicating that as a widely expressed protein in mammalian cells, BRD2’s biological role is not only limited to transcriptional regulation, but also plays an important role in maintaining genomic stability.

4. The role of BRD2 in diseases

4.1 Malignant tumors

As a co-regulatory factor for gene transcription, mutations in BRD2 are associated with the occurrence, progression, apoptosis, and drug resistance in various types of tumors (Figure 4). For instance, in colorectal cancer, the BRD2/acetylated ELK4 complex can modulate the expression of laminin subunit beta 3 (LAMB3), suppress FOXO3/4 expression via the AKT-FOXO3/4 axis, thereby diminishing the tumor-inhibitory effect of FOXO3/4 (Table 2) (Zhu, Song et al., 2020). In prostate cancer, BRD2 can mediate chromatin remodeling downregulated androgen receptors, thereby promoting prostate cancer resistance to castration therapy (Urbanucci, Barfeld et al., 2017). BRD2 can also promote malignant tumor cell proliferation, migration, and stress response by regulating epithelial mesenchymal transition (EMT) in lung cancer cells (Serresi., Kertalli. et al., 2021). BRD2 can also inhibit Fas mediated cell apoptosis by regulating the expression of melanoma apoptotic protein Livin (Sugihara, Hashimoto et al., 2020). BRD2 can promote chemotherapy resistance in adult T-lymphocytic leukemia by activating the RasGRP1-Ras-EPK signaling pathway (Tian, Cai et al., 2020). BRD2 induces acquired resistance to MEK inhibitors in ovarian epithelial cancer through kinase reprogramming (Kurimchak, Shelton et al.,...
BRD2 promotes the expression of immune checkpoint PD-L1 in head and neck squamous cell carcinoma cells, leading to drug resistance in malignant tumor cells (Bhola, Njatcha et al., 2021). In addition, mutations or abnormal expression of BRD2 can also serve as molecular markers to predict the genetic susceptibility and prognosis of malignant tumors (Liu, Goldstein et al., 2019; Perez-Pena, Paez et al., 2019; Yu, Hsu et al., 2019; Jafari, Kolla et al., 2021).

Although BRD2 is involved in the occurrence and development of several malignant tumors, it does not play a promoting role in all tumor types. For example, according to the TCGA database, higher expression of BRD2 is positively associated with better overall survival in patients with renal clear cell carcinoma. Moreover, significant differences in BRD2 expression levels are detected among different tumor types according to the TCGA database. These data indicate the functional heterogeneity of BRD2 in tumor occurrence and development, and further research is needed to investigate the specific role of BRD2 in different tumor types.

4.2 Neurological disorders

Recent studies have revealed that BRD2 is a risk gene associated with Parkinson's disease (PD), and α-Synuclein can induce BRD2 expression, thereby contributing to neuroinflammation and the onset of Parkinson's disease (Sarkar, Dammer et al., 2020). After the acute systemic inflammation was induced by lipopolysaccharide in the hippocampus of mice, the expression levels related to epigenetic regulation (histone deacetylase (Hdac4, 5, 8, 9, 11) and BRD3) were downregulated, while BRD2 was upregulated (Czapski, Zhao et al., 2020). Juvenile myoclonic epilepsy (JME) is an idiopathic epilepsy that occurs during adolescence and mainly caused by genetic factors. In recent years, it has been found that BRD2 may be a susceptibility gene for JME (Gilsoul, Grisar et al., 2019). The high methylation modification of the BRD2 promoter region may be related to JME (Pathak, Miller et al., 2018; El-Osta, 2019), but some researchers have questioned this conclusion (Schulz, Ruppert et al., 2019).

4.3 Inflammatory and immune diseases
BRD2 can promote the assembly of histone acetylation dependent transcriptional complexes through the BD domain, thereby regulating the expression of various inflammatory genes, indicating the importance of BRD2 in regulating inflammatory response (Liu, Yang et al., 2021). Interleukin 17A (IL-17A) plays a crucial role in the development of many autoimmune diseases such as psoriasis. Knocking down the expression of BRD2 reduces the expression of CXC motif chemokine 1/2/6 (CXCL1/2/6) and granulocyte colony stimulating factor (G-CSF) mediated by IL-17A/TNF. In BRD2-deficient keratinocytes, many genes crucial for keratinization and homeostasis are dysregulated. The expression level of rheumatoid arthritis related genes is down regulated (Slivka, Hsieh et al., 2019). Deep hypothermic circulatory arrest is a measure to reduce oxygen consumption in the body, especially in the brain. It is commonly used in complex cardiovascular surgery, but postoperative neuroinflammation can lead to neurological damage. After the deep hypothermic circulatory arrest, BRD2-NKκB signaling pathway can aggravate neuronal damage by upregulating cold-induced RNA binding proteins and promoting the release of inflammatory cytokines (Liu, Li et al., 2020). BRD2 plays an important role in upregulating the inflammatory response of NK cells and promotes the expression and secretion of IFNγ (Cribbs, Filippakopoulos et al., 2021). In bone marrow-derived macrophages from Brd2 knockout (Brd2-/-) mice, the stimulation of TNFα by LPS is inhibited (Belkina, Nikolajczyk et al., 2013). BRD2 may be a susceptibility gene for rheumatoid arthritis and inflammatory bowel disease (Ostrowski, Paziewska et al., 2016; Zhu, Xia et al., 2016; Pernat Drobez, Repnik et al., 2018).

4.4 Metabolic diseases

With the progress of material metabolism, the body obtains the energy needed to sustain life activities from sugars, fats, and proteins. Type 2 diabetes patients generally have white adipose tissue dysfunction, and excessive free fatty acid release has adverse effects on lipid metabolism, causing insulin resistance. Under physiological conditions, BRD2 is highly expressed in the pancreatic islets β cells, thereby inhibiting mitosis and insulin transcription (Wang, Liu et al., 2009). As an important energy reservoir in the body, Adipose tissue is crucial for maintaining
normal metabolism. BRD2 is also an important transcription regulatory factor for adipocyte differentiation (Zang, Wang et al., 2013). Overexpression of BRD2 in white adipose tissue can mediate insulin resistance in mice through the mTOR/Akt signaling pathway (Sun, Wu et al., 2017). In white adipose tissue of mice, overexpression of BRD2 promotes the expression of lipolysis related genes, while inhibiting the expression of fat synthesis related genes. Overexpression of BRD2 activates hormone sensitive lipase expression, inhibits ERK-dependent cyclolipoid 1, reduces the size of white adipose tissue and promotes the release of free fatty acids and glycerol in plasma. All these regulations are related to adipolysis (Zong, Li et al., 2019). In addition, overexpression of BRD2 can also cause the expression of NF-κB target genes to increase pro-inflammatory and chemotactic factors in adipose tissue (Sun, Wu et al., 2017). Knocking out BRD2 in adipose tissue can promote the conversion of white adipose tissue into brown adipose tissue, a process known as “browning” (Bagchi, Ferguson et al., 2018). These results indicate that BRD2 plays an important regulatory role in energy metabolism in the body.

4.5 Virus infection

BRD2's role in viral infection is multifaceted. In terms of antiviral immunity, the expression of type I interferon stimulated genes (ISGs) requires the interaction between the transcription factor complex ISGF3 and the target gene promoter to initiate transcription and prevent infection. BRD2 can regulate interferon mediated transcription and antiviral immune induced H2A.Z inhibition (Au-Yeung and Horvath, 2018). The important role of BRD2 in gene transcription makes it suitable as a target for the proliferation of certain viruses.

Kaposi's sarcoma associated virus (KSHV) can cause Kaposi's sarcoma, primary effusion lymphoma, and certain Castleman diseases. KSHV's LANA (kLANA) is the first viral protein found to interact with members of the BET family. kLANA plays an important role in viral replication, fixing the viral genome onto cell chromosomes, and regulating viral and cellular gene transcription (PLATT., SIMPSON. et al., 1999; Garber, Hu et al., 2002; Barbera, Chodaparambil et al., 2006). KSHV has a stable incubation period in B lymphocytes and pleural effusion lymphoma. During the
incubation period, the KSHV genome is a segment where gene expression is inhibited. Recent studies have found that BRD2 and BRD4 can be localized in some regions of the KSHV virus genome, including the LANA binding site within the terminal repeat TR, as well as the CTCF Cohesion site in the latent and activation control regions, thereby maintaining the chromatin structural stability of the KSHV incubation period (Zhang, Kwok-Shing Ng et al., 2017). The ET domain of BRD2 is the main target of most retroviral proteins to interact, such as murine leukemia virus MLV, porcine endogenous retrovirus A/C (PERV A/C), Rauss associated virus type 1 (RAV-1), and HIV-1 (Weidner-Glunde., Ottinger. et al., 2010; Aiyer, Swapna et al., 2014; Gallay, Blot et al., 2019). The structural protein E of COVID-19 (SARS-CoV-2) can interact with the BD domain of BRD2, altering the gene expression in host cells and creating an environment favorable for virus replication (Gordon, Jang et al., 2020). In addition, the main receptor angiotensin converting enzyme 2 (ACE2) that SARS CoV-2 enters host cells is also regulated by BRD2 protein transcription (Samelson, Tran et al., 2022). These findings suggest that BRD2 may be an effective target for the treatment of COVID-19 infection.

5. Summary and Outlook

Serving as a pivotal factor in epigenetic modifications, BRD2 orchestrates the recruitment of transcription factors and co-stimulatory/inhibitory factors to fine-tune the transcriptional regulation of target genes. BRD2 is involved in the occurrence and development of various diseases, and is associated with MYC and NF-κB. The RAS and RUNX signaling pathways, closely intertwined with BRD2, have emerged as promising therapeutic targets garnering considerable attention. Nevertheless, our comprehension of BRD2 remains limited, and its precise functions in both physiological and pathological contexts are still far from being fully elucidated. Furthermore, studies have revealed that systemic knockout of BRD2 results in embryonic lethality in mice, highlighting the potential side effects associated with BRD2 inhibitors.

Broad-spectrum inhibitors targeting the BET family have found widespread utility in fundamental scientific research. While certain small molecule drugs aimed at
BET proteins have demonstrated promising effects in early-stage clinical trials, none have yet transitioned into clinical practice. Meanwhile, members of the BET family also have significant functional heterogeneity. At present, PROTAC technology has greatly improved the targeting of a certain member of the BET family, but it still cannot avoid the inhibitory effect on other members. In addition, most BET family targeted inhibitor development has focused on BRD4 and less on BRD2. A substantial body of evidence underscores the significant role of BRD2 in disease pathogenesis. Consequently, there remains a compelling need for ongoing research efforts aimed at the design and development of highly selective BRD2 inhibitors with enhanced activity.

**Abbreviations**

ACE2, angiotensin converting enzyme 2; ATAC, Assay for Targeting Accessible Chromatin; BD, bromodomain; BET, Bromodomain and extra-terminal domain; BRD2, Bromodomain and extra-terminal domain protein 2; CDK9, cyclin-dependent kinases 9; CXCL1/2/6, CXC motif chemokine 1/2/6; CTD, C-terminal motif; EMT, epithelial mesenchymal transition; ET, external domain; G-CSF, granulocyte colony stimulating factor; HAT, histone acetyltransferase; HDAC, histone deacetylase; IL-17A, Interleukin 17A; ISGs, type I interferon stimulated genes; JME, Juvenile myoclonic epilepsy; KAT, lysine acetyltransferase; KAT7, lysine acetyltransferase 7; KSHV, Kaposi’s sarcoma associated virus; LAMB3, laminin subunit beta 3; PCNA, proliferating cell nuclear antigen; PD, Parkinson's disease; PERV A/C, porcine endogenous retrovirus A/C; PROTAC, Protein Degradation Targeting Complexes; P-TEFb, positive transcription elongation factor b; RAV-1, Rauss associated virus type 1.

**Author’s contributions**

Wrote or contributed to the writing of the manuscript: YJ, WC and XW.

**Data Availability Statement**
The reported structures of BET protein family members (Figure 1) are openly available in Pfam database. The figures 1, 3 and 4 are created with BioRender.com. All other data presented are contained within the manuscript data.

Declaration of competing interest

The authors declare that they have no competing interests.

Footnotes

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Figure 1. The structure details of BET protein family members.

Figure 2. Schematic illustration of the specialized E3 ligases for potential PROTAC applications.
The tissue specificities of E3 ligases may be used to promote the PROTAC applications to target different tumor types (Békés, Langley et al., 2022). Copyright 2022, The Proceedings of Springer Nature Limited.

Figure 3. Schematic illustration of the biological functions of BRD2.
BRD2 involves in biological process by three ways, including (A) regulating transcription, (B) forming chromatin spatial structure, and (C) maintaining genomic stability.

Figure 4. Schematic illustration of the association of BRD2 with different diseases.
BRD2 involves in many diseases, such as malignant tumor, inflammatory diseases, neurological disorders, metabolic diseases, and virus infection.
References:


Czapski, G. A., Y. Zhao, W. J. Lukiw and J. B. Strosznajder (2020). Acute Systemic Inflammatory Response Alters Transcription Profile of Genes Related to Immune Response and Ca(2+) Homeostasis in Hippocampus; Relevance to Neurodegenerative


Garber, A. C., J. Hu and R. Renne (2002). Latency-associated nuclear antigen (LANA) cooperatively binds to two sites within the terminal repeat, and both sites contribute to the ability of LANA to suppress transcription and to facilitate DNA replication. J Biol Chem 277: 27401-27411.


Antitumor Activity in Gastric Cancer via MYC-Targets and G2M-Checkpoint Signaling Pathways. *Frontiers in Oncology* 11.


Patel, K., P. D. Solomon, J. L. Walshe, D. J. Ford, L. Wilkinson-White, R. J. Payne, J. K. K.


for post-myeloproliferative neoplasm secondary AML. *Leukemia* 33: 1373-1386.


(PROTAC) exerts potent lethal activity against mantle cell lymphoma cells. *Leukemia* 32: 343-352.


Zong, J., S. Li, Y. Wang, W. Mo, R. Sun and M. Yu (2019). Bromodomain-containing
### Table 1 Summary of BET protein proteolysis targeting chimera in development.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Action</th>
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<tr>
<td>dBET1</td>
<td>Leukemia</td>
<td>[40, 41]</td>
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<tr>
<td></td>
<td>Colorectal cancer</td>
<td>[44]</td>
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<tr>
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<td></td>
<td>Lung cancer</td>
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<td>Myeloproliferative neoplasms</td>
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<td></td>
<td>Glioblastoma</td>
<td>[32]</td>
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<td></td>
<td>Adenoid cystic carcinoma</td>
<td>[49]</td>
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<td>BETd-260</td>
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<td>[73]</td>
</tr>
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<td></td>
<td>Osteosarcoma</td>
<td>[74]</td>
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<td></td>
<td>Acute leukemia</td>
<td>[35]</td>
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<tr>
<td></td>
<td>Hepatocellular carcinoma</td>
<td>[145]</td>
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<tr>
<td>MZ1</td>
<td>Colorectal cancer</td>
<td>[44]</td>
</tr>
<tr>
<td></td>
<td>Esophageal squamous cell carcinomas</td>
<td>[75]</td>
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<td></td>
<td>Breast cancer</td>
<td>[36, 76]</td>
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<tr>
<td></td>
<td>Lung cancer</td>
<td>[77]</td>
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<tr>
<td></td>
<td>Melanoma</td>
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<td>QCA570</td>
<td>Leukemia</td>
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<td>Bladder cancer</td>
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<td></td>
<td>Lung cancer</td>
<td>[81]</td>
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<td>A1874</td>
<td>Osteosarcoma, Lung cancer, Melanoma,</td>
<td>[82]</td>
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<td>Leukemia</td>
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<td></td>
<td>Colon cancer</td>
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<td>ARV-771</td>
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<td></td>
<td>Mantle cell lymphoma</td>
<td>[53]</td>
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<tr>
<td></td>
<td>Post-myeloproliferative neoplasm secondary AML</td>
<td>[54, 55]</td>
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<td></td>
<td>Prostate cancer</td>
<td>[56]</td>
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<td></td>
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<td></td>
<td>Hepatocellular Carcinoma</td>
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<td>ARV-825</td>
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<td>Liposarcoma</td>
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<td></td>
<td>Acute myelogenous leukemia</td>
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<td>Glioma</td>
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<td></td>
<td>T-cell acute lymphoblastic leukemia</td>
<td>[64, 65]</td>
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<td>Post-myeloproliferative neoplasm secondary AML</td>
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<td>Mantle cell lymphoma</td>
<td>[53]</td>
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<td></td>
<td>Breast cancer</td>
<td>[36, 66]</td>
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<tr>
<td></td>
<td>Colorectal cancer</td>
<td>[67]</td>
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<td></td>
<td>Multiple myeloma</td>
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<td>Pancreatic cancer</td>
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<td>Thyroid carcinoma</td>
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<td>Gastric cancer</td>
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<td></td>
<td>Melanoma</td>
<td>[72]</td>
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<td>SIM1</td>
<td>Lung cancer, Leukemia</td>
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Table 2 The role of BRD2 in different diseases.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>The role of BRD2 in diseases</th>
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<tbody>
<tr>
<td>Colorectal cancer</td>
<td>The BRD2/acetylated ELK4 complex modulates the expression of laminin subunit beta 3 (LAMB3), suppress FOXO3/4 expression via the AKT-FOXO3/4 axis, thereby diminishing the tumor-inhibitory effect of FOXO3/4 (Zhu, Song et al., 2020).</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>BRD2 mediates chromatin remodeling downregulated androgen receptors, thereby promoting resistance to castration therapy (Urbanucci, Barfeld et al., 2017).</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>BRD2 promotes tumor cell proliferation, migration, and stress response by regulating epithelial mesenchymal transition (EMT) (Serresi., Kertalli. et al., 2021).</td>
</tr>
<tr>
<td>Tumor</td>
<td>BRD2 inhibit Fas mediated cell apoptosis by regulating the expression of apoptotic protein Livin (Sugihara, Hashimoto et al., 2020).</td>
</tr>
<tr>
<td>Melanoma</td>
<td>BRD2 promotes chemotherapy resistance by activating the RasGRP1-Ras-EPK signaling pathway (Tian, Cai et al., 2020).</td>
</tr>
<tr>
<td>Adult T-lymphocytic leukemia</td>
<td>BRD2 induces acquired resistance to MEK inhibitors through kinase reprogramming (Kurimchak, Shelton et al., 2019).</td>
</tr>
<tr>
<td>Ovarian epithelial cancer</td>
<td>BRD2 promotes the expression of immune checkpoint PD-L1, leading to drug resistance in tumor cells (Bhola, Njatcha et al., 2021).</td>
</tr>
<tr>
<td>Head and neck squamous cell carcinoma</td>
<td>The α-Synuclein can induce BRD2 expression, thereby contributing to neuroinflammation and the onset of Parkinson's disease (Sarkar, Dammer et al., 2020).</td>
</tr>
<tr>
<td>Parkinson's disease</td>
<td>The high methylation modification of the BRD2 promoter region may be related to JME (Pathak, Miller et al., 2018; El-Osta, 2019).</td>
</tr>
<tr>
<td>Neurological disorders</td>
<td></td>
</tr>
<tr>
<td>Juvenile myoclonic epilepsy</td>
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<tr>
<td>Inflammatory and immune diseases</td>
<td>Knocking down the expression of BRD2 reduces the expression of CXC motif chemokine 1/2/6 (CXCL1/2/6) and granulocyte colony stimulating factor (G-CSF) mediated by IL-17A/TNF (Slivka, Hsieh et al., 2019).</td>
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</tbody>
</table>

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<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
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<tr>
<td>BRD2 plays an important role in upregulating the inflammatory response of NK cells and promotes the expression and secretion of IFNγ (<a href="#">Cribbs, Filippakopoulos et al., 2021</a>).</td>
<td></td>
</tr>
<tr>
<td>In bone marrow-derived macrophages from Brd2 knockout (Brd2−/−) mice, the stimulation of TNFα by LPS is inhibited (<a href="#">Belkina, Nikolajczyk et al., 2013</a>).</td>
<td></td>
</tr>
<tr>
<td>In BRD2-deficient keratinocytes, the expression level of rheumatoid arthritis related genes is down regulated (<a href="#">Slivka, Hsieh et al., 2019</a>).</td>
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<tr>
<td>Rheumatoid arthritis</td>
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<tr>
<td>Deep hypothermic circulatory arrest</td>
<td>BRD2-NKxB signaling pathway can aggravate neuronal damage by upregulating cold-induced RNA binding proteins and promoting the release of inflammatory cytokines after the deep hypothermic circulatory arrest (<a href="#">Liu, Li et al., 2020</a>).</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>BRD2 may be a susceptibility gene for inflammatory bowel disease (<a href="#">Ostrowski, Paziewska et al., 2016; Zhu, Xia et al., 2016; Pernat Drobez, Repnik et al., 2018</a>).</td>
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<tr>
<td>Metabolic diseases</td>
<td>Type 2 diabetes</td>
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<tr>
<td>Kaposi's sarcoma associated virus (KSHV)</td>
<td>BRD2 is localized in some regions of the KSHV virus genome, thereby maintaining the chromatin structural stability of the KSHV incubation period (<a href="#">Zhang, Kwok-Shing Ng et al., 2017</a>).</td>
</tr>
<tr>
<td>Virus infection</td>
<td></td>
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<tr>
<td>SARS-CoV-2</td>
<td>The structural protein E of COVID-19 (SARS-CoV-2) can interact with the BD domain of BRD2, altering the gene expression in host cells and creating an environment favorable for virus replication (<a href="#">Gordon, Jang et al., 2020</a>). The main receptor angiotensin converting enzyme 2 (ACE2) that SARS CoV-2 enters into host cells is also regulated by BRD2 protein transcription (<a href="#">Samelson, Tran et al., 2022</a>).</td>
</tr>
</tbody>
</table>
### Figure 2

CNS-enriched E3s
- TRIM9
- RNF182
- Many others

Tumour-enriched E3s
- DCAF2
- TRIM71
- MAGE5
- Many others

Muscle-enriched E3s
- KLHL41
- KLHL40

Ubiquitous E3s
- CRBN
- VHL
- GID4
- Many others

<table>
<thead>
<tr>
<th>E3 ligase family</th>
<th>Example member</th>
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<th>Tumour-dependent</th>
<th>Tissue-enriched</th>
<th>Insights into ubiquitylation mechanism exist</th>
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<tr>
<td>ELOB/ELOC</td>
<td>VHL</td>
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<td>No</td>
<td>No</td>
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<tr>
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<td>CRBN</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Kannt &amp; Dikic (2021)</td>
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<td>CTLH</td>
<td>GID4</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<td>DCAF</td>
<td>DCAF2</td>
<td>Yes</td>
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<td>TRIM</td>
<td>TRIM9</td>
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<td>SPRY</td>
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<td>Ishida &amp; Ciulli (2021)</td>
</tr>
</tbody>
</table>
Figure 3

A. Regulating transcription

B. Forming chromatin spatial structure

C. Maintaining genomic stability

Illustrations include complex molecular interactions involving proteins such as BRD2, TF, Cohesion, CTCF, RNAPII, and Top1, along with DNA structures such as target genes, DSB (DNA double-strand break), and DNA damage.
Figure 4

Malignant tumor

BRD2

progression

therapy resistance

rheumatoid arthritis
psoriasis
inflammatory bowel disease
Parkinson's disease
JME
diabetes

Inflammatory diseases
Neurological disorders
Metabolic diseases
virus infection