Cellular Immunology 337 (2019) 48–53

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Review article

Emerging roles of and therapeutic strategies targeting BRD4 in cancer

Mary E. White[a], Joelle M. Fenger[b], William E. Carson III[c,*]

[a] Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus OH, United States
[b] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus OH, United States
[c] Department of Surgery, Division of Surgical Oncology, The Ohio State University Wexner Medical Center and The OSU James Comprehensive Cancer Center, Columbus, OH, United States

ARTICLE INFO

Keywords:
Bromo- and Extra-Terminal (BET) protein family
BRD4
Inflammation and cancer
BET inhibitors

ABSTRACT

The Bromodomain and Extra-terminal (BET) family of proteins were first recognized as important epigenetic regulators in inflammatory processes; however, there is increasing evidence to support the notion that BET proteins also play a critical role in 'reading' chromatin and recruiting chromatin-regulating enzymes to control gene expression in a number of pathologic processes, including cancer. To this end, the mechanisms by which BET proteins regulate chromatin remodeling and promote tumor-associated inflammation have been heavily studied over the past decade. This article to review the biology of BET protein dysfunction in promoting tumor-associated inflammation and cancer progression and the application of small molecule inhibitors that target specific BET proteins, alone or in combination with immunomodulatory agents as a novel therapeutic strategy for cancer patients.

1. Introduction

1.1. BET bromodomain proteins

Epigenetic modifications are reversible, heritable alterations to the DNA of a cell that do not involve a change in the nucleotide sequence. A variety of epigenetic mechanisms, including changes in CpG island methylation patterns and histone modifications regulate gene expression and play an important role in maintaining normal cellular homeostasis. Dysregulation of proteins responsible for interacting with and modifying macro-molecular complexes of DNA and histone proteins called chromatin is frequently observed in inflammatory cells and cancer cells, supporting the idea that epigenetic regulation of gene expression contributes to disease pathogenesis [1,2]. One family of proteins that function as chromatin "readers" and recruit chromatin-modifying enzymes to gene target promoters are the bromodomain-containing proteins. Bromodomains are highly conserved domains that are made up of approximately 110 amino acids and are composed of a left-handed bundle of four alpha helices, which are linked by ZA and BC loops that vary in sequence between the bromodomain proteins. Despite variations in the ZA and BC loops, amino acid residues involved in acetyl lysine binding including asparagine (Asn) and tyrosine (Tyr) are conserved in bromodomains [3–6]. Bromodomain-containing proteins function as primary readers of acetylated lysine residues on the N-terminal tails of histones [7]. Sixty-one bromodomains (BrD) are present in the human genome representing eight subfamilies that classify members within the group. This review will focus on members belonging to subfamily II, the Bromo- and Extra-Terminal domain (BET) protein family [3–6].

The BET family is composed of four proteins (BRD2, BRD3, BRD4 and testis-specific BRDT) that contain two conserved, tandem bromodomain motifs (BD1 and BD2). BD1 and BD2 contain a hydrophobic pocket that binds acetylated lysine residues present on histones and/or transcription factors. Once bound, BET bromodomain proteins recruit and activate positive transcription elongation factor–b (P–TEFb). Upon activation, P-TEFb phosphorylates serine 2 (S2) on the carboxyl-terminal domain of RNA Pol II, which is required for transcription elongation [4] (Fig. 1). The most extensively studied member of the BET family of proteins is BRD4, which is the only ubiquitously expressed BET family member that directly binds P-TEFb [1,6]. In health, the BRD4 protein is required for maintenance of chromatin stability and controls the transition of cells from M phase to G1 phase during cell cycling, in part, through recruitment of P-TEFb [8]. *In vivo* studies demonstrate that heterozygous *Brd4*$^{+/-}$ mice have severe defects in cell differentiation and organogenesis and *Brd4*-null animals die *in utero* [8], indicating that BRD4 is required for normal cell cycle progression and cellular development.

* Corresponding author at: Division of Surgical Oncology, Department of Surgery, The Ohio State University, 410 W 10th Ave, N911 Doan Hall, Columbus, OH 43210-1267, United States.
E-mail address: William.Carson@osumc.edu (W.E. Carson).

https://doi.org/10.1016/j.cellimm.2019.02.001
Received 17 January 2019; Received in revised form 27 January 2019; Accepted 2 February 2019
Available online 04 February 2019
0008-8749/

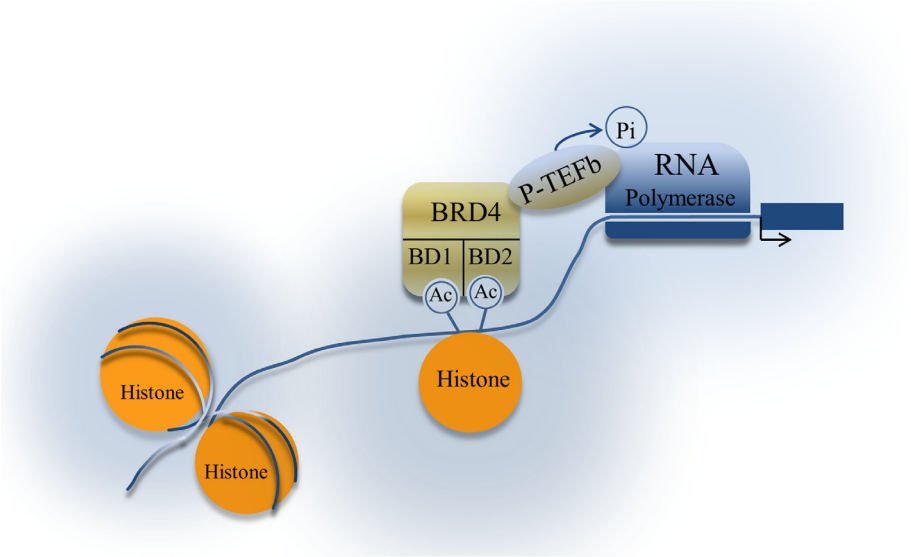

Fig. 1. BRD4 regulation of transcription. BET proteins bind acetylated lysine residues on histones and recruit positive transcription elongation factor–b (P–TEFb). P-TEFb phosphorylates serine 2 on the carboxyl-terminal domain of RNA Polymerase, which is required for transcription elongation.

2. Mechanisms and consequences of BRD4 dysregulation in cancer

The BET family of proteins were initially recognized for their role as important epigenetic regulators in inflammation and inflammatory diseases; however, it is now well established that BET proteins are frequently deregulated in cancer and contribute to aberrant chromatin remodeling and gene transcription that mediates tumorigenesis [9,10]. *BRD4* gene rearrangements or *BRD4* gene mutations including missense substitutions and nonsense substitutions have been documented in a number of human cancers [11]. Aberrant expression of BET proteins, specifically BRD4, promotes the progression of cell cycling, invasion and metastasis of cancer cell lines *in vitro*. For example, gene fusion events in squamous cell carcinoma cells involving *BRD4* (or *BRD3*) and the nuclear protein in testes (*NUT*) gene enhances cell invasion and migration *in vitro* and is associated with a highly aggressive variant of this cancer, known as NUT midline carcinoma in people[12]. Similarly, amino acid substitutions predominantly localized to residues in the two terminal helices αB and αC and proximal to the acetyl- lysine binding site of BRD4 promote the oncogenic properties of BRD4 [13]. Lori et al. found that amino acid substitutions involving these regions in the BET family of proteins altered tertiary protein structure and decreased protein stability at high temperatures. Taken together, these findings suggest that genetic events affecting BET family members may alter protein conformation and impact protein–protein or protein-DNA interactions that regulate biological processes that mediate to tumor initiation, progression and metastasis [13].

3. Role of BRD4 in promoting inflammation and cancer initiation

Tumor initiation is the first step in tumor development and is the process by which normal cells undergo malignant transformation. Numerous reports have demonstrated a strong association between chronic inflammation induced by metabolic or infectious etiologies with malignant cellular transformation and tumor initiation [14,15]. In the process of clearing infectious agents and normal wound healing, chronic inflammatory conditions promote cellular activation, replication, and may impair DNA damage repair processes or epigenetic regulatory mechanisms resulting in the transformation and propagation of a neoplastic cell population. Studies have demonstrated an increased incidence of breast cancer in humans with type 2 diabetes (T2D) and found that the presence of inflammatory cell infiltrates in the neoplastic microenvironment is associated with shorter disease-free survival in breast cancer patients [16]. These data suggest that visceral adipose tissue (VAT) inflammation, which is frequently present in patients with T2D, may lead to chronic inflammation of the breast adipose tissue and the induction of pro-inflammatory cytokines such as IL-6, TNFα, IL-17A and IL-22 that promote cancer initiation. In support of this, the expression of RORC nuclear binding protein, which is essential for adipocyte development and Th17 *T*-lymphocyte differentiation, increases during obesity and up-regulates IL-17A, IL-17F and IL-22 transcript expression [17]. Binding of BRD4 to the *RORC* promoter directly enhances IL-17 and IL-22 transcript expression and this was reversed by targeted inhibition of BRD4, providing a potential mechanism by which BRD4 may control the expression of pro-inflammatory cytokines through epigenetic regulation of *RORC*.

The *c-MYC* oncogene encodes for MYC, a transcription factor that has broad effects on cell cycle progression, apoptosis and the establishment and maintenance of pluripotency. Alterations in MYC expression and function are common in both inflammatory and neoplastic conditions suggesting that MYC regulates critical molecular and cellular pathways that link chronic inflammation to tumorigenesis [18]. *In vivo* murine studies demonstrate that mice with increased VAT [19] have enhanced MYC nuclear activity and high circulating levels of fibroblast-growth factor 2 (FGF2) which promotes epithelial cell transformation in the skin and colon [20]. Targeted inhibition of BRD4 using small molecule inhibitors attenuates VAT volume, FGF2 release, and blocks the neoplastic transformation of epithelial cells, in part, by inhibiting MYC-dependent transcription [19].

Cellular senescence is a mechanism that maintains cellular homeostasis, induces cell-cycle arrest in damaged cells, and prevents pre-malignant cell propagation and transformation. Senescent cells normally maintain a closed chromatin configuration; however, cancer cells can acquire a secretory-associated senescent phenotype (SASP) whereby oncogene-induced senescence results in remodeling of the enhancer landscape and recruitment of BRD4 [21]. Consequently, BRD4 enhances the expression of SASP factors such as IL-1α, IL-1β, IL-8, BMP2 and INHBA [21,22]. Targeted inhibition of BRD4 using BET inhibitors blocks the expression of SASP factors and induces senescence in cancer cells, resulting in enhanced recognition and phagocytosis of cancer cells by NK cells and M1 polarized macrophages [21].

4. BET protein family members enhance tumor-associated inflammation and progression

Tumor progression is defined as the acquisition of phenotypic properties such as enhanced cell growth and invasiveness in a neoplastic cell population [23]. Several mediators such as NF-κB, Cox-2, MYC, cyclin D1 and CD47 have established roles in promoting inflammation and activating pro-inflammatory signaling pathways within the tumor microenvironment that influence tumor progression. NF-κB plays dual roles in cancer. NF-κB is involved in activation of host innate immune responses; however constitutive activation of NF-kB is a key feature of many cancers and NF-κB-mediated signaling promotes the transcription of pro-tumorigenic and pro-inflammatory cytokines such as IL-6 and TNF-α [24]. The BET family of proteins have established roles in regulating the expression of inflammatory cytokines implicated in promoting the development and progression of cancer. For example, studies have shown that BRD4 binds to the acetylated lysine-310 residue on RelA resulting in co-activation and stabilization of NF-κB in human lung carcinoma cell lines [25]. Concordantly, BRD2 has a demonstrated role in regulating the activity of NF-κB in melanoma cell lines, suggesting a conserved role for bromodomain-containing proteins in controlling NF-κB pathway activation [26]. Targeting of this pathway using pan-BET inhibitors decreases the activation of NF-κB by inhibiting NF-κB associated protein, p50, and its precursor, p105. Inhibition of BRD2 decreases the growth of melanoma cells by blocking NF-κB-dependent signaling and results in inhibition of NF-κB target gene expression, including IL-6, IL-8, VEGF, CCL5 and CXCL10. Moreover, pharmacologic inhibition of the pro-tumorigenic cytokines IL-6 and IL-8 blocks this autocrine signaling loop and decreases NF-κB activation [26].

Throughout the course of cancer progression, cancer cells acquire additional properties such as immunoevasion or chemotherapeutic resistance that is in part, mediated by molecular cross-talk between cancer cells and neighboring tumor-associated stromal and inflammatory cells in the tumor microenvironment. To this end, studies have identified a role for BET proteins in regulating the expression of the transcription factor MYC and demonstrate that therapeutic targeting of BRD4 inhibits BRD4 binding to the *c-MYC* promoter and prevents the expression of MYC-dependent target genes in both cancer cells and inflammatory cell populations present in the tumor microenvironment [27–30]. In KRAS-driven cancers, up-regulation of MYC increases the expression of CCL9 and IL-23 [31]. CCL9 is a chemotactic factor involved in the recruitment of CD206+ macrophages to the tumor microenvironment and enhances the production and release of cytokines that promote angiogenesis and aberrant tumor blood vessel formation. In contrast, IL–23 is a cytokine that suppresses the functions of tumor-infiltrating effector T cells, B cells and NK cells, thereby promoting tumor immunoevasion [31]. These data highlight the potential role of BRD4 in regulating signaling networks in both cancer cells and the surrounding tumor microenvironment and suggest that therapeutic targeting of BET proteins may represent a novel strategy to inhibit BRD4-mediated pathways in tumor cells and tumor-associated stroma that promote cancer progression (Fig. 2).

5. Dysregulation of BET proteins promotes inflammation and enhances the metastatic phenotype of cancer cells

Metastasis is the distant migration, via lymph or blood, of malignant cells from the primary tumor site to other tissues. A defining feature of aggressive, metastatic cancer cells is the development of chemotherapeutic resistance that significantly influences outcomes in cancer patients. During the process of epithelial to mesenchymal transition (EMT) cancer cells lose their cell polarity, cell–cell adhesions, and gain invasive properties that initiate the metastatic cascade. Signaling through the Jagged1/Notch1 pathway promotes EMT and enhances the invasive phenotype in breast cancer cells. Jagged1 is a Notch ligand and

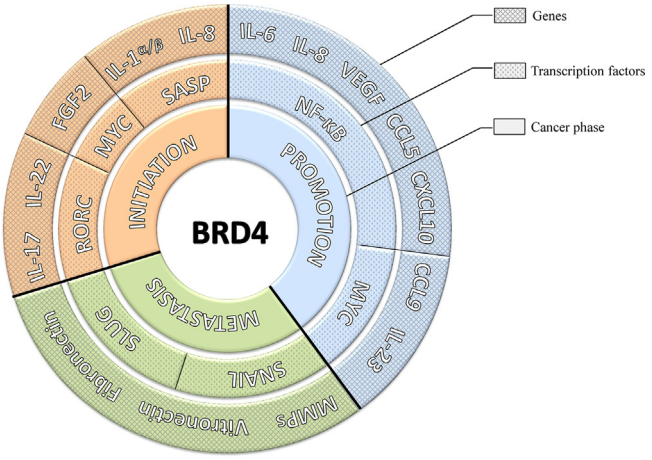

Fig. 2. BRD4 dysregulation promotes cancer initiation, progression and metastasis. Aberrant expression of BRD4 can alter transcription factors and genes that affect all aspects of cancer.

upon binding of Jagged1 to the Notch receptor, Notch is cleaved and translocates to the nucleus where it binds to CBF1, Suppressor of Hairless, Lag-1 (CSL) and Coenzyme A (CoAs) and activates the expression of transcription factors that are vital in EMT, such as Snail and Slug [32]. Studies have shown that BRD4 promotes the migratory properties of breast cancer cell lines through Notch1-induced signaling [15,33]. Interestingly, studies have demonstrated that the pro-inflammatory cytokine IL-6 increases BRD4 enrichment at the proximal portion of the Jagged1 promoter region, supporting the idea that tumor-associated inflammatory mediators may alter the epigenetic 'reading' functions of BRD4 in cancer cells. To this end, treatment of triple negative breast cancer cell lines with the BET protein inhibitor, JQ1 reduces cellular invasion and migration and reduces IL-6 mediated signaling through the Jagged1/Notch1 pathway. These findings provide insight into the role of BRD4 in epigenetic regulation of gene pathways that promote the metastatic phenotype of cancer cells and suggest that therapies targeting BRD4 may represent a strategy to prevent cancer metastasis.

6. Epigenetic regulation of gene expression by BRD4 in the tumor microenvironment

Tumors are composed of a heterogenous population of cells, which includes both neoplastic tumor cells and non-neoplastic cells that create the tumor microenvironment (TME). The TME is composed of numerous cell types including immune cells, fibroblasts, bone marrow-derived inflammatory cells, and endothelial cells that support tumor blood vessel growth. Within the TME, there is continuous signaling and molecular cross talk that occurs between tumor cells and cells that have been recruited to the microenvironment. Collectively, the molecular signaling events that occur within the TME function to promote tumor progression and enable cancer cells to acquire phenotypic properties such as enhanced invasion and migration that are critical to the development of cancer metastasis. In support of this, recent data suggests a direct role for BRD4 in the epigenetic regulation of gene expression in both cancer cells and non-neoplastic cells present in the TME that promotes tumorigenesis [15,34].

Immune cells are a major component of the tissue microenvironment and they produce a variety of cytokines and chemokines that directly affect cancer cell behavior leading to tumor growth and metastasis. For example, tumor-associated macrophages present within the tumor microenvironment influence the aggressive metastatic behavior of a number of solid malignancies [35]. Macrophages can be classified based on their activation patterns as "M1" or "M2" (or "classic" and "alternative") macrophages, wherein M1 macrophages are pro-

inflammatory and M2 macrophages are anti-inflammatory [18]. In the context of cancer, M2-like macrophages are considered pro-tumorigenic as this phenotype inhibits the innate immune response directed against tumor cells [36]. The STAT3 transcription factor plays an important role in activating signaling pathways that promote the differentiation of macrophages towards the M2 phenotype [37] and inhibition of p-STAT3 signaling in pancreatic ductal adenocarcinoma cells results a decrease in M2 polarized macrophages [18]. Similarly, therapeutic targeting of BET proteins using the small molecule inhibitor JQ1 increases the secretion of IL-12β, a cytokine that possesses anti-tumor and anti-angiogenic activity. Concordant with this finding, treatment with JQ1 decreases M2 macrophage activation and reduces the secretion of the angiogenic factor, VEGF from tumor cells [18,38].

Stromal cells that reside within the tumor microenvironment also provide a source of pro-tumorigenic cytokines, chemokines and growth factors that enhance cancer cell cycle progression and alter the tumor immune environment. In breast cancer, stromal cells within the tumor microenvironment express significantly higher levels of cyclin D1 protein compared to non-tumor control tissue [39]. Cyclin D1 expression is regulated by the NF-κB and MYC transcription factors and studies have demonstrated that pharmacologic inhibition of BET proteins decreases Cyclin D1 expression in a MYC-dependent fashion [18,27,40]. Cyclin D1 promotes the production of pro-inflammatory cytokines and chemokines including CCL2 CCL7, CCL11 CXCL5, CXCL9, CXCL12 GM-CSF, CXCL1, TNFα and TNF-β suggesting a potential mechanism by which stromal cells may promote the aggressive nature of breast cancers [39]. Moreover, the increase in cyclin D1-dependent cytokine and chemokine expression enhances the recruitment and infiltration of CD34-positive hematopoietic stem cells into tumor tissues and promotes their differentiation to CD11b-positive myeloid derived suppressor cells (MDSCs) [39]. These data suggest that BRD4-mediated signaling in tumor-associated stromal and inflammatory cells may play an important role in regulating gene networks that promote the recruitment of MDSCs and enhance the immunoevasive properties of cancer cells.

7. Therapeutic strategies to target BET bromodomain proteins in cancer

Numerous studies have implicated the BET bromodomain family of proteins in regulating biological processes such as inflammation and have demonstrated that BET protein dysregulation contributes to the initiation, progression and metastatic behavior of cancer cells [41]. Importantly, maintenance of the malignant phenotype in cancer cells is, in part, dependent on epigenetic deregulation in both hematopoietic and solid tumor cells [42]. This dependence upon epigenetic proteins makes for a promising therapeutic target. As such, therapies aimed at targeting epigenetic regulators and inhibiting BET protein function may represent a novel therapeutic strategy for cancer. Several small molecule inhibitors of BET proteins have been developed and demonstrate anti-tumor properties *in vitro*. Furthermore, human phase I clinical trials evaluating the safety and efficacy of novel, small molecule inhibitors of BET proteins demonstrate that BET inhibitors exhibit minimal and reversible clinical toxicity in human cancer patients [43]. Both *in vitro* and *in vivo* studies with BET inhibitors have confirmed target inhibition and exhibit potential therapeutic effects; however, phase I clinical trials conducted in human cancer patients using BET inhibitors have failed to show a significant therapeutic benefit, suggesting that BET inhibition may have limited success as a single agent therapy [44].

8. Combination strategies involving targeted inhibitors of BET proteins

While BET inhibitors have shown little therapeutic benefit for use as a single agent, a number of studies have demonstrated that BET inhibitors exhibit synergistic anti-tumor activity when combined with other small molecule inhibitors or immunotherapies [45]. In this section, we will focus on novel combination strategies involving small molecule inhibitors of BET proteins and immunomodulatory drugs for anti-cancer treatment.

Lenalidomide and pomalidomide are potent immunomodulatory drugs (IMiDs) that are frequently used in the treatment of hematopoietic malignancies such as multiple myeloma. Studies evaluating the effect of lenalidomide in primary effusion lymphoma (PEL) show that lenalidomide up-regulates interferon-α, β, and γ expression and is directly cytotoxic to PEL cells. When used in combination with a BRD4 inhibitor, the anti-proliferative effects of lenalidomide on cancer cell lines is significantly enhanced [43,46,47]. Studies have demonstrated that the combination therapy using a BRD4 inhibitor and lenalidomide acts synergistically to decrease tumor burden and increase overall survival times and that this mediated in part, by inhibition of BRD4-dependent MYC activation along with the cytotoxic effects of IMiDs [46].

Bruton's tyrosine kinase (BTK) is a cytoplasmic tyrosine kinase that regulates B-cell proliferation, survival and chemotaxis and is frequently dysregulated in B-cell malignancies [48]. In normal B lymphocytes, antigen-specific activation of the B cell receptor (BCR) initiates a signaling cascade that involves BTK and results in B-cell proliferation and survival. BTK is also involved in non-BCR mediated signaling pathways and can undergo activation via alternative cell surface receptors (e.g. Toll-like receptors, growth factor receptors and chemokine receptors CXCR4 and CXCR5) that regulate chemotaxis and tissue homing [48]. In neoplastic B-cells, BTK inhibitors work synergistically when combined with BET inhibitors. Dual targeting of BET proteins and BTK in malignant B-cells down-regulates MYD88 expression resulting in decreased activation of MYD88-dependent signaling through NF-κB, Toll-like receptors, and the JAK/STAT pathway [43].

PD-L1, the ligand for PD-1, plays a critical role in the regulation of T cell immune checkpoint activation. Overexpression of PD-L1 has been documented in a variety of human malignancies and there are now a number of clinical trials evaluating the safety and efficacy of immune checkpoint inhibitors alone or in combination with other therapies in cancer. Interestingly, Zhu et al. [34] found that BRD4 is a critical regulator of PD-L1 expression in tumor cells, myeloid dendritic cells (DC) and macrophages. BRD4 directly binds to the CD274 (encoding PD-L1) gene promoter to activate its transcription. In murine cancer models, treatment with the BET inhibitor JQ1 significantly down-regulated PD-L1 expression in tumor cells and tumor-associated immune cells and correlated with increased anti-tumor activity of cytotoxic T cells [1,34]. In MYC-driven lymphomas, combination therapy with JQ1 and PD-1 monoclonal antibodies substantially decreased tumor burden and prolonged overall survival when compared to either therapeutic agent alone [49].

There is growing interest in utilizing adoptive immunotherapeutic strategies such as tumor-specific chimeric antigen receptor (CAR)-expressing *T*-cells in cancer. However, the use of CAR-T cell therapy has in part, been limited by the induction of antigen-specific toxicities affecting normal tissues and adverse side effects of CAR-T cell treatments such as life-threatening cytokine-release syndromes. In subsets of patients undergoing CAR-T cell therapy, this treatment fails to completely eradicate tumor cells [50]; therefore, rational strategies involving CAR-T cells in combination with other therapies is being explored with the goal of preventing cancer relapse. One such strategy involves treating patient-derived CD8+ T cells prior to adoptive transfer with the pan-BET family inhibitor, JQ1. In a study conducted by Kagoya et al. [51], JQ1-treated CD8+ T cells maintain stem cell-like memory (Tscm) and central memory (Tcm) and demonstrate superior anti-tumor properties compared to effector memory T cells (Tem). Furthermore, treatment with JQ1 inhibits BRD4 binding at the *BATF* gene promoter, thereby inhibiting T effector cell differentiation [51]. Subsequent adoptive transfer of JQ1-treated CD8+ T cells in murine leukemia models

resulted in increased overall survival times, indicating that pre-treatment with BET inhibitors may represent a strategy to improve the efficacy of adoptive T cell immunotherapy.

Lastly, strategies involving dual inhibition of PI3K and BRD4-mediated signaling pathways have been evaluated in MYC-driven cancers. Whereas BRD4 is a known epigenetic regulator of MYC gene transcription, signaling through the PI3K pathway prevents degradation of the MYC protein. Morpholinothienopyrane (SF2523) is a dual small molecular inhibitor of PI3K and BRD4 that has demonstrated biological activity in orthotopic murine models of high-risk *MYCN*-amplified neuroblastoma. Treatment with SF2523 decreased neuroblastoma tumor volume and reduced metastatic disease burden, in part by inhibiting MYC expression and activation, which suggests that dual targeting of PI3K and BRD4 may represent an alternative strategy to enhance therapeutic response in MYC-driven cancers [27].

9. Conclusions

There is a growing body of evidence documenting the association of chronic inflammation with cancer development and it is well established that pro-inflammatory conditions promote cancer initiation, progression and metastasis. Deregulation of epigenetic readers, writers and erasers are widely implicated as playing key roles in the pathogenesis of inflammatory conditions and cancer, thereby providing a mechanistic link between the two biological processes. The family of BET proteins functions as epigenetic readers and regulates the expression of genes that promote inflammation and malignant cellular transformation. Pharmacologic inhibition of BET family members results in chromatin remodeling and repression of pro-inflammatory genes and oncogenes (MYC, NF-κB, and cyclin D1, among others) that drive tumor-associated inflammation and tumor progression. This is supported by *in vivo* and *in vitro* studies demonstrating that therapeutic targeting of BET family proteins decreases tumor burden and improves survival times in murine models of cancer. Novel small molecule inhibitors of BET proteins exhibit minimal clinical toxicity; however, they have shown limited success as single-agents in Phase I clinical trials in human cancer patients. Combination strategies involving BET inhibitors and immunomodulatory drugs demonstrate synergistic activity by virtue of their direct effect on tumor cells and indirectly by blocking pro-inflammatory signaling in stromal and inflammatory cells present in the tumor microenvironment. Future studies characterizing the role of BET protein family members in regulating genes that promote inflammation and neoplastic transformation will provide a foundation for the rational design and therapeutic targeting of BET proteins and other pro-inflammatory mediators, with the ultimate goal of improving outcomes in cancer patients.

Acknowledgement

This work was supported by NIH grant: T32 CA090223.

References

[1] R. Prinjha, A. Tarakhovsky, Chromatin targeting drugs in cancer and immunity, Genes Dev. 27 (16) (2013) 1731–1738.
[2] D. Bayarsaihan, Epigenetic mechanisms in inflammation, J. Dent. Res. 90 (1) (2011) 9–17.
[3] N. Zaware, M.M. Zhou, Chemical modulators for epigenome reader domains as emerging epigenetic therapies for cancer and inflammation, Curr. Opin. Chem. Biol. 39 (2017) 116–125.
[4] M.J. Barrero, Epigenetic Strategies to Boost Cancer Immunotherapies, Int. J. Mol. Sci. 18 (6) (2017).
[5] B.N. Devaiah, A. Gegonne, D.S. Singer, Bromodomain 4: a cellular Swiss army knife, J. Leukoc. Biol. 100 (4) (2016) 679–686.
[6] M. Perez-Salvia, M. Esteller, Bromodomain inhibitors and cancer therapy: from structures to applications, Epigenetics 12 (5) (2017) 323–339.
[7] S. Muller, P. Filippakopoulos, S. Knapp, Bromodomains as therapeutic targets, Expert Rev. Mol. Med. 13 (2011) 1–21.
[8] M.R. Schweiger, et al., Brd4-independent transcriptional repression function of the papillomavirus e2 proteins, J. Virol. 81 (18) (2007) 9612–9622.
[9] J.T. Deeney, et al., BET bromodomain proteins Brd2, Brd3 and Brd4 selectively regulate metabolic pathways in the pancreatic beta-cell, PLoS One 11 (3) (2016) e0151329.
[10] I. Marazzi, et al., Chromatin dependencies in cancer and inflammation, Nat. Rev. Mol. Cell Biol. 19 (4) (2018) 245–261.
[11] J.E. Bradner, D. Hnisz, R.A. Young, Transcriptional addiction in cancer, Cell 168 (4) (2017) 629–643.
[12] C.A. French, Small-molecule targeting of BET proteins in cancer, Adv. Cancer Res. 131 (2016) 21–58.
[13] L. Lori, et al., Effect of BET missense mutations on bromodomain function, inhibitor binding and stability, PLoS One 11 (7) (2016) e0159180.
[14] S.I. Grivennikov, M. Karin, Inflammation and oncogenesis: a vicious connection, Curr. Opin. Genet. Dev. 20 (1) (2010) 65–71.
[15] G.P. Andrieu, et al., BET proteins in abnormal metabolism, inflammation, and the breast cancer microenvironment, J. Leukoc. Biol. (2018).
[16] D.A. Nicholas, et al., BET bromodomain proteins and epigenetic regulation of inflammation: implications for type 2 diabetes and breast cancer, Cell. Mol. Life Sci. 74 (2) (2017) 231–243.
[17] A. Nadeem, et al., Imiquimod-induced psoriasis-like skin inflammation is suppressed by BET bromodomain inhibitor in mice through RORC/IL-17A pathway modulation, Pharmacol. Res. 99 (2015) 248–257.
[18] A.S. Leal, et al., Bromodomain inhibitors, JQ1 and I-BET 762, as potential therapies for pancreatic cancer, Cancer Lett. 394 (2017) 76–87.
[19] D. Chakraborty, et al., A BET bromodomain inhibitor suppresses adiposity-associated malignant transformation, Cancer Prev. Res. (Phila) 11 (3) (2018) 129–142.
[20] D. Chakraborty, et al., Fibroblast growth factor receptor is a mechanistic link between visceral adiposity and cancer, Oncogene 36 (48) (2017) 6668–6679.
[21] N. Tasdemir, et al., BRD4 connects enhancer remodeling to senescence immune surveillance, Cancer Discov. 6 (6) (2016) 612–629.
[22] S. Watanabe, et al., Impact of senescence-associated secretory phenotype and its potential as a therapeutic target for senescence-associated diseases, Cancer Sci. 108 (4) (2017) 563–569.
[23] D.J. McConkey, et al., Molecular genetics of bladder cancer: Emerging mechanisms of tumor initiation and progression, Urol. Oncol. 28 (4) (2010) 429–440.
[24] V. De Simone, et al., Th17-type cytokines, IL-6 and TNF-alpha synergistically activate STAT3 and NF-kB to promote colorectal cancer cell growth, Oncogene 34 (27) (2015) 3493–3503.
[25] Z. Zou, et al., Brd4 maintains constitutively active NF-kappaB in cancer cells by binding to acetylated RelA, Oncogene 33 (18) (2014) 2395–2404.
[26] S.J. Gallagher, et al., Control of NF-kB activity in human melanoma by bromodomain and extra-terminal protein inhibitor I-BET151, Pigment Cell Melanoma Res. 27 (6) (2014) 1126–1137.
[27] F.H. Andrews, et al., Dual-activity PI3K-BRD4 inhibitor for the orthogonal inhibition of MYC to block tumor growth and metastasis, Proc. Natl. Acad. Sci. U.S.A 114 (7) (2017) E1072–E1080.
[28] C.J. Ott, et al., BET bromodomain inhibition targets both c-Myc and IL7R in high-risk acute lymphoblastic leukemia, Blood 120 (14) (2012) 2843–2852.
[29] V. Posternak, M.D. Cole, Strategically targeting MYC in cancer. F1000Res, 2016. 5.
[30] J.A. Mertz, et al., Targeting MYC dependence in cancer by inhibiting BET bromodomains, Proc. Natl. Acad. Sci. U.S.A 108 (40) (2011) 16669–16674.
[31] R.M. Kortlever, et al., Myc cooperates with ras by programming inflammation and immune suppression, Cell 171 (6) (2017) p. 1301-+.
[32] A. Garcia, J.J. Kandel, Notch: a key regulator of tumor angiogenesis and metastasis, Histol. Histopathol. 27 (2) (2012) 151–156.
[33] G. Andrieu, et al., BRD4 regulates breast cancer dissemination through Jagged1/Notch1 signaling, Cancer Res. 76 (22) (2016) 6555–6567.
[34] H. Zhu, et al., BET bromodomain inhibition promotes anti-tumor immunity by suppressing PD-L1 expression, Cell Rep 16 (11) (2016) 2829–2837.
[35] S. Singh, et al., Initiative action of tumor-associated macrophage during tumor metastasis, Biochim. Open 4 (2017) 8–18.
[36] S. Aras, M.R. Zaidi, TAMeless traitors: macrophages in cancer progression and metastasis, Br. J. Cancer 117 (11) (2017) 1583–1591.
[37] N. Wang, H.W. Liang, K. Zen, Molecular mechanisms that influence the macrophage M1–M2 polarization balance, Front. Immunol. 5 (2014).
[38] W.B. Liu, et al., Tumor-derived vascular endothelial growth factor (VEGF)-A facilitates tumor metastasis through the VEGF-VEGFR1 signaling pathway, Int. J. Oncol. 39 (5) (2011) 1213–1220.
[39] T.G. Pestell, et al., Stromal cyclin D1 promotes heterotypic immune signaling and breast cancer growth, Oncotarget 8 (47) (2017) 81754–81775.
[40] C.Y. Zhang, et al., Epigenetic blockade of neoplastic transformation by bromodomain and extra-terminal (BET) domain protein inhibitor JQ-1, Biochem. Pharmacol. 117 (2016) 35–45.
[41] S.I. Grivennikov, F.R. Greten, M. Karin, Immunity, inflammation, and cancer, Cell 140 (6) (2010) 883–899.
[42] J.S. Roe, et al., BET bromodomain inhibition suppresses the function of hematopoietic transcription factors in acute myeloid leukemia, Mol. Cell 58 (6) (2015) 1028–1039.
[43] M. Boi, et al., The BET bromodomain inhibitor OTX015 affects pathogenetic pathways in preclinical B-cell tumor models and synergizes with targeted drugs, Clin. Cancer Res. 21 (7) (2015) 1628–1638.
[44] A. Stathis, F. Bertoni, BET proteins as targets for anticancer treatment, Cancer Discovery 8 (1) (2018) 24–36.
[45] D.B. Doroshow, J.P. Eder, P.M. LoRusso, BET inhibitors: a novel epigenetic approach, Ann. Oncol. 28 (8) (2017) 1776–1787.
[46] R. Gopalakrishnan, et al., Immunomodulatory drugs target IKZF1-IRF4-MYC axis in

primary effusion lymphoma in a cereblon-dependent manner and display synergistic cytotoxicity with BRD4 inhibitors, Oncogene 35 (14) (2016) 1797–1810.
[47] A. Moros, et al., Synergistic antitumor activity of lenalidomide with the BET bromodomain inhibitor CPI203 in bortezomib-resistant mantle cell lymphoma, Leukemia 28 (10) (2014) 2049–2059.
[48] J.A. Burger, Bruton's tyrosine kinase (BTK) inhibitors in clinical trials, Curr. Hematol. Malig. Rep. 9 (1) (2014) 44–49.
[49] S.J. Hogg, et al., BET-Bromodomain Inhibitors Engage the Host Immune System and Regulate Expression of the Immune Checkpoint Ligand PD-L1, Cell Rep. 18 (9) (2017) 2162–2174.
[50] D.A. Mele, et al., BET bromodomain inhibition suppresses TH17-mediated pathology, J. Exp. Med. 210 (11) (2013) 2181–2190.
[51] Y. Kagoya, et al., BET bromodomain inhibition enhances T cell persistence and function in adoptive immunotherapy models, J. Clin. Invest. 126 (9) (2016) 3479–3494.