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BET bromodomain proteins and epigenetic regulation of inflammation: implications for type 2 diabetes and breast cancer

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Abstract

Chronic inflammation drives pathologies associated with type 2 diabetes (T2D) and breast cancer. Obesity-driven inflammation may explain increased risk and mortality of breast cancer with T2D reported in the epidemiology literature. Therapeutic approaches to target inflammation in both T2D and cancer have so far fallen short of the expected improvements in disease pathogenesis or outcomes. The targeting of epigenetic regulators of cytokine transcription and cytokine signaling offers one promising, untapped approach to treating diseases driven by inflammation. Recent work has deeply implicated the Bromodomain and Extra-Terminal domain (BET) proteins, which are acetylated histone “readers”, in epigenetic regulation of inflammation. This review focuses on inflammation associated with T2D and breast cancer, and the possibility of targeting BET proteins as an approach to regulating inflammation in the clinic. Understanding inflammation in the context of BET protein regulation may provide a basis for designing promising therapeutics for T2D and breast cancer.

Keywords

Chromatin reader; Metabolism

Introduction

The increasing prevalence of obesity, not only in the US, but worldwide, has become an alarming public health concern. Approximately one-third of adults in the US are obese, and the rise in obesity is paralleled by the increasing prevalence of T2D and obesity-associated cancers, such as breast cancer [1]. It is imperative to understand mechanisms that link obesity to increased risk of T2D and co-morbid cancer to develop approaches that

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specifically address this risk. Unequivocal epidemiological data associate obesity with both diabetes and cancer [2–4]. Several studies have provided evidence that obesity per se is not the driving factor. Rather, the metabolic and inflammatory status of individuals is proposed to be the most important factor for disease risk [3, 5]. Based on the literature, chronic inflammation is clearly implicated in the pathogenesis of type 2 diabetes (T2D) and cancer [6–13]. Nevertheless, targeting this inflammation in T2D has not been effective [14–17] and targeting inflammation in cancer progression is yet to be thoroughly explored. Novel approaches to characterize and target inflammation in these disease processes will be necessary to overcome prior limitations. The previous approaches to reduce inflammation in T2D include neutralizing antibodies to individual cytokines thought to be important for disease progression [14–17]. However, obesity-induced inflammation is not likely driven by a single cytokine [18]. In cancer, epidemiological studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit cyclooxygenase (COX), reduce breast cancer risk [19]. The anti-inflammatory COX-2 inhibitor celecoxib (Celebrex) shows an anti-tumor transcriptional response in primary breast cancer as well as preliminary efficacy in clinical trials for advanced breast cancer [19, 20].

One under-utilized approach to treating chronic inflammation in disease is to target epigenetic regulators of cytokine transcription and signaling. The BET family of “histone reader” proteins plays critical roles in inflammation [5, 21–23], suggesting that the three somatic proteins of this understudied family have potential to become useful targets for therapeutic regulation of inflammation in obesity-associated T2D, and subsequent T2D-associated cancers. Not only do BET proteins regulate inflammation, they also control cell cycle, a useful direct target in cancer (reviewed in [5]). The purpose of this review is to focus on inflammation associated with T2D and breast cancer, and the possibility of targeting BET proteins as a clinical approach to regulating unresolved, chronic inflammation.

Inflammation in obesity-associated type 2 diabetes

Understanding pro-inflammatory immune cells and their corresponding cytokine networks as mediators of T2D provides a basis to determine promising therapeutic targets. Obesity is the leading risk factor associated with the development of T2D. Obesity results in multiple inflammatory events. Some of these events include adipose tissue hypoxia [24, 25], leptin secretion and unfolded protein responses that are activated by endoplasmic reticulum stress [26]. Secretion of cytokines and increased lipolysis are also characteristic of obesity-driven inflammation [27]. Visceral adipose tissue (VAT) has proven to be a key site for the inflammation that is associated with T2D [28]. Infiltration of VAT by immune cells results in low-grade chronic inflammation and secretion of pro-inflammatory cytokines that can induce insulin resistance (IR), both locally and systemically (reviewed in [29]).

Immune cells, including macrophages, B cells and T cells, contribute to cytokine networks associated with diabetogenic inflammation and the development of T2D pathology [6, 28, 30–36]. Macrophages are, by far, the best studied immune cells in diabetogenic inflammation. Macrophages are antigen-presenting cells (APCs) deemed responsible for activating T cells and secreting the bulk of cytokines during chronic inflammation in T2D [37–39]. The infiltration (or in situ differentiation) of the so-called ‘M1’ pro-inflammatory

macrophages into adipose tissue during obesity contributes to cytokine networks through secretion of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, IL-12, resistin, chemokine ligand 5 (CCL5), and CCL2/MCP-1 [40, 41]. Similarly, B cells contribute to inflammation in T2D, although B cells are significantly less well studied than macrophages (reviewed in [42]). Much of what is known about the role of B cells in T2D inflammation is derived from diet-induced obese (DIO) mice studies. During obesity in mice, the total number of B cells increases in VAT [43], although in human obesity, few B cells are found in VAT depots [44]. Transfer of B cells from DIO mice to DIO B cell-null mice deteriorates the metabolic health of the recipient. Conversely, B-cell-deficient DIO mice have increased insulin sensitivity [43, 45]. However, B-cell induction of IR seems to be largely T-cell-dependent; studies show that transfer of splenic B cells from DIO mice to DIO RAG1^{null} mice, which lack both T and B cells, has little effect on glucose tolerance [8]. Winer et al. demonstrated that B-cell-deficient mice have reduced levels of CD8⁺ T cells that produce interferon (IFN) γ in the fat [43]. T cells from humans with T2D only produce disease-associated levels of IL-17 in the presence of B cells [8]. Studies, such as these, identify both T cells and B cells as important for diabetogenic inflammation.

T cells and their roles in diabetogenic inflammation have also been extensively studied in mice (reviewed in [46]). In general, DIO mice have three times more T cells in VAT than mice fed a control diet which suggests increased T-cell recruitment [47]. CD4⁺ T cells co-localize with clusters of macrophages and dying (apoptotic/necrotic) adipocytes called “crown-like” structures (CLS) [48], which are also found in inflamed human breast adipose tissue [10, 25, 40, 49]. T-cell depletion reduces VAT inflammation, glucose intolerance, and IR in DIO mice [50]. The importance of T-cell infiltration in obesity-driven IR is highlighted by the fact that inhibition of T-cell migration to VAT prevents IR in DIO mice [51]. The pro-inflammatory T cell subsets most commonly implicated in T2D inflammation in mice are Th1 cells and CD8⁺ T cells [52–55]. However, multiple investigations indicate that Th17 cells may be the central T-cell subset in human diabetogenic inflammation [56, 57]. Th17 cells are a pro-inflammatory subset of T helper cells (characterized by secretion of IL-17A, IL-17F, and IL-6) that frequently associate with autoimmunity and inflammatory diseases [58, 59]. Only monocytes from obese individuals with T2D (as opposed to lean individuals with T2D) support IL-17 production from T cells in vitro [60]. The disproportionate importance of Th17 mechanisms in human T2D was most convincingly shown by recent work that identified a pro-inflammatory Th17 cytokine signature, which differentiates subjects with T2D from metabolically healthy subjects, irrespective of BMI [18], and predicts T2D status with higher than random fidelity.

Although analysis of inflammation in obesity-driven T2D has generally relied on the analysis of individual cytokines, our recent work highlights the importance of analyzing cytokine networks. Analyses focused on circulating T cells, as sources of T2D-associated inflammation, identify a dominant Th17 cell cytokine profile, not just one cytokine, as important to differentiate equally obese subjects without diabetes (ND) and T2D subjects [18]. The existence of a cytokine network, as opposed to a single dominant cytokine, that drives inflammation in T2D may be the reason clinical trials that neutralize single pro-inflammatory “diabetogenic” cytokines, such as TNF- α or IL-1 β [9, 11, 61–69], have yielded disappointing results [14–17]. We propose that, to target effects of a pathogenic

cytokine network, therapies that simultaneously target leading regulatory pathways are necessary, for example, epigenetic regulation of cytokine gene transcription.

Defining new therapeutic targets for inflammation-mediated development of T2D in humans requires the ability to study diabetogenic inflammation in large cohorts. Although adipose tissue is an important site of inflammation in subjects with T2D, large-scale analysis with adipose tissue is both limiting and limited. Several studies have demonstrated that circulating immune cells is a valid alternative to adipose immune cells to study diabetogenic inflammation [7, 43, 70–73]. One example of similarities between human adipose tissue and circulating immune cells is that the ratio of inflammatory Th1 and Th17 cells to anti-inflammatory regulatory T cells is higher in obese/IR compared with obese/insulin sensitive subjects [7, 70–73]. A clear definition of diabetogenic inflammation will allow for the design of effective anti-inflammatory approaches that could also be useful for diabetes-associated cancers, such as breast cancer.

Inflammation as a risk factor in breast cancer

Epidemiological studies have associated increased risk and mortality in breast cancer with T2D [74–81]. Women with T2D have ~20 % increased risk of breast cancer compared with women without T2D [74]. The Cancer Prevention Study II, among others [74–77, 82], found that the incidence of T2D was associated with increased risk of mortality in women [hazard ratio (HR) (1.16) (95 % confidence interval (CI) (1.03–1.29)] [77]. Men with diabetes have an even greater risk of mortality [4.20 (2.20–8.04)] from breast cancer than females [77]. One obvious risk factor that may link T2D and breast cancer is obesity-induced diabetogenic inflammation [9–11, 61–69]. Because inflammation in blood and adipose tissue depots is correlated [10, 69, 83], cytokine networks identified in obesity-associated T2D likely reflect local cytokine networks present in breast adipose tissue and could potentially explain increased mortality from breast cancer in patients with T2D. Although a causal relationship between T2D and breast cancer has yet to be established in humans, specific mouse models have begun to solidify this connection. Santander et al. demonstrated that DIO mice with breast tumors have larger tumors and increased numbers of CLS in breast adipose tissue compared to lean mice with breast tumors [84]. In obesity resistant BALB/c female mice, high fat diet is pro-tumorigenic for ER-breast cancer cell lines. The high fat diet increases breast cancer cell proliferation, lung and liver metastasis, angiogenesis, and circulation levels of pro-tumorigenic factors, such as VEGF, IL-6, and leptin [85]. Two other mouse studies have been able to link metabolic changes to inflammation and breast cancer progression. Feeding conjugated linoleic acid (CLA) to mice increases pro-inflammatory macrophage infiltration into adipose tissue [86]. When fed to ovariectomized PyMT mice, CLA increases mammary ductal hyperplasia and is associated with hyperinsulinemia [87]. In humans, breast adipose tissue inflammation and T2D are associated with shorter disease-free breast cancer survival [10, 74–81]. Thus, if obesity and T2D increase breast adipose tissue inflammation similar to VAT inflammation, it is possible that diabetogenic inflammation could contribute directly to breast cancer progression via the inflammatory microenvironment (Fig. 1). It will be important for future studies to determine the mechanistic role of obesity and T2D in human breast cancer progression. In summary,

targeting inflammation in subjects with both T2D and breast cancer to ameliorate breast cancer aggressiveness is an important avenue to explore for the development of therapeutics.

Because T2D associates with worse breast cancer outcomes [77], we suspect that diabetogenic inflammation is a critical factor that contributes to these outcomes. Inflammation associates with breast cancer progression and mortality in several human studies [10, 88–91]. Chronic inflammation is accepted to be carcinogenic and T2D in women associates with increased mortality, as well as adipose tissue inflammation in subjects who develop breast cancer [10]. This research suggests that diabetogenic inflammation may produce an inflammatory microenvironment in breast adipose tissue that could promote breast cancer, and likely exacerbates tumor progression. Various cytokines have been implicated in breast cancer aggressiveness (reviewed in [92–94]). One such class is the Th17-associated cytokines IL-17A and IL-22. Increased intratumoral IL-17 mRNA in humans is associated with increased aggressiveness of invasive ductal carcinoma, reduced disease-free survival, and worse prognosis [95]. IL-17⁺ cells are identified in the microenvironment of breast cancer sections [96] and tumor-infiltrating T cells in mice produce increasing amounts of IL-17 as the tumor advances [95]. Interestingly, IL-17 may play multiple roles during cancer progression by acting directly on breast cancer cells, but also by remodeling the breast cancer microenvironment, to elicit tumor-associated angiogenesis, immune system evasion, and metastasis [97, 98]. IL-17A induces tumor growth in vivo and suppresses apoptosis [95]. The in vitro addition of IL-17 to the cell lines MDA-MB-231 and MDA-MB-435 markedly increases Matrigel invasion [96]. In further support of a role for Th17-driven inflammation in breast cancer progression, the systemic neutralization of IL-17A reduces breast cancer metastasis to the bone and lungs in arthritic mice [99, 100]. In addition, IL-17-producing immune cells are associated with tumor progression in breast cancer patients [101, 102], as well as tumor growth and angiogenesis in a mouse model of breast cancer [103]. IL-17 promotes resistance to anti-angiogenic therapy, such as anti-VEGF antibody treatment [104]. Finally, IL-17 produced by $\gamma\delta$ T cells drives neutrophil polarization into a ‘myeloid-derived suppressor cell-like’ phenotype, leading to inhibition of cytotoxic CD8⁺ T cells and eliciting metastasis [105]. These insights strongly support a mechanistic link between the Th17 cytokine profile in T2D and breast cancer outcomes.

BET protein regulation of inflammation

Inflammatory processes, such as those identified in T2D and breast cancer, are subject to epigenetic regulation. Transcriptional control through epigenetic mechanisms includes the activity of chromatin “reader”, “writer”, and “eraser” proteins (reviewed in [106, 107]). One such family of proteins, the Bromodomain and extra-terminal domain (BET) proteins, is widely implicated in epigenetic regulation of inflammation (reviewed in [5, 21–23]). The BET proteins, comprised of three somatic proteins, bromodomain-containing protein (BRD)2, BRD3, BRD4; and BRDT, a testis-specific variant, contain two mutually exclusive bromodomains (reviewed in [21]). The bromodomain is a 110-amino acid structural motif that gives BET proteins the ability to bind *N*- ϵ -acetylated lysine on histones in chromatin, and, therefore, to “read” histone tails in nucleosomal chromatin. This interaction enables BET proteins to recruit transcription co-regulators to promoters, thereby to control important

genes, such as genes that are critical for inflammation, cell death, cell cycle, and proliferation in both normal and disease states [108, 109]. The bromodomain structure is highly conserved among species [108]. Humans have about 42 bromodomain-containing proteins [110, 111], which include scaffolding proteins, histone acetyltransferases, and helicases. Hydrogen bonding, often at asparagine residues, is the primary biochemical interaction that bromodomains utilize to bind to acetylated histones [112]. Thus, these structural units are fundamental, ubiquitous, and critical for transcription as effectors of signal transduction and as regulators of inflammatory disease.

The multiple mechanisms by which BET proteins regulate gene transcription have been well studied for BRD4 (reviewed in [113]). BRD4 enhances gene transcription through RNA Polymerase II (Pol II) by both direct and indirect mechanisms. Although the biochemical mechanisms are not understood, BRD4 exhibits intrinsic kinase activity that phosphorylates Pol II at serine-2 [114]. The intrinsic kinase activity of BRD4 is also important for the function of topoisomerase I. Phosphorylation of the carboxyl terminal domain (CTD) of Pol II by BRD4 is necessary for the CTD stimulation of topoisomerase I to induce DNA relaxation during Pol II-driven transcription [115]. BRD4 also promotes Pol II elongation by recruiting active positive transcriptional elongation factor b (P-TEFb) to phosphorylate Pol II [116, 117]. P-TEFb, a heterodimer of cyclin-dependent kinase 9 (Cdk9) and one of its regulatory subunits, Cyclin T1, T2, or K, is normally associated with 7SK/HEXIM, a ribonucleoprotein complex that sequesters P-TEFb in an inactive state. BRD4 binding to P-TEFb interrupts this association and recruits P-TEFb to acetylated chromatin [116]. In addition to promoting elongation, BRD4 is known to recruit transcription factors (TFs) to regulate gene transcription. An *in vitro* biochemical screen performed by Wu et al. identified c-Jun, p53, YY1, AP2, C/EBP α , V/EBP β , and the Myc/Max heterodimer as TFs that interact with BRD4 in an acetylation-independent manner [118]. Interestingly, BRD4 can also interact with acetyl groups on TFs, for example, TWIST [119]. Like BRD4, the two other somatic family members BRD2 and BRD3 also promote gene transcription via association with hyperacetylated chromatin. In addition, BRD2 has intrinsic histone chaperone activity [120]. Importantly for inflammation, BRD4 is a coactivator of NF- κ B. BRD4 binds to acetylated RelA via its bromodomains and recruits CDK9 to phosphorylate Pol II. These bromodomain-dependent interactions enhance the transcription of NF- κ B-dependent pro-inflammatory cytokine genes [121]. The ability of BET proteins to regulate NF- κ B, a regulator of inflammatory pathways, makes this family of proteins an appealing target for design of epigenetically directed therapeutic interventions in diabetogenic inflammation and breast cancer.

BET protein control of cytokine production

NF- κ B is a master regulator of inflammation through its ability to regulate transcription of numerous cytokine genes. BET protein inhibitors have been shown to regulate general cytokine production through inhibition of recruitment of transcriptional machinery to gene promoter regions, and interruption of NF- κ B target gene transcription [122–124]. Overall, pan-BET inhibitors are very effective at blocking NF- κ B-driven secretion of pro-inflammatory cytokines through various mechanisms (Table 1). In general, BET protein inhibition blocks the expression of the NF- κ B target genes (Table 1) induced by mediators,

such as TNF- α and bacterial lipopolysaccharide (LPS) [122]. Not only does JQ1 block NF- κ B target gene transcription, but JQ1 also prevents phosphorylation of I κ B, the upstream negative regulator of NF- κ B [122]. In human macrophages, LPS activation of toll-like receptor 4 (TLR4) results in increased association of BRD4 with gene promoter regions [125, 126], whereas treatment with I-BET ablates this association [125]. Inhibition of BET proteins with I-BET151 inhibits the recruitment of transcriptional machinery, such as CREB-binding protein (which interacts with the NF- κ B complex), to the IL-6 promoter [127].

In addition to regulating NF- κ B signaling, BET proteins also regulate the STAT signaling pathway [125, 127]. STATs are important cytokine signaling mediators utilized by various immune cell receptors [128]. Deacetylase inhibitors are known to repress STAT5-mediated transcription [103, 129]. However, the mechanism of this inhibition was only recently suggested to be via displacement of BET proteins from acetylated target genes. Global non-specific acetylation induced by deacetylase inhibitors may dilute the effect of BET proteins [130]. In support of this concept, these authors also demonstrated that BRD2 associates with transcriptionally active STAT5 target genes. Moreover, JQ1 inhibits the expression of STAT5 target genes [130]. The previous work demonstrating that JQ1 reduces phosphorylation of JAK2/STAT5 supports the interpretation that BET proteins regulate STAT5 signaling [131]. In addition to STAT5, studies performed in primary human macrophages implicate BET proteins in the regulation of STAT1-mediated transcription [125]. These authors established that the pan-BET bromodomain inhibitor I-BET151 inhibits LPS-induced transcription and, more interestingly, inhibits cytokine-induced transcription of STAT target genes in a gene-specific manner [125]. These data clearly demonstrate that BET protein regulation of inflammation is not an all-or-none phenomenon, but rather a pleiotropic process that regulates specific genes, depending on the stimuli and particular cell type.

There are multiple demonstrations that BET protein inhibition decreases cytokine production (Table 1), including IL-6 (pleiotropic), IL-17, and IL-1 β , which are cytokines implicated in both diabetogenic inflammation and breast cancer [122, 132–134]. In 2010, the demonstration that the small molecule inhibitor JQ1 associates with the hydrophobic pocket of bromodomains to interrupt histone binding [110], and that I-BET762, a synthetic acetylated histone mimic, disrupts BET protein interaction with chromatin, suggested that JQ1 and I-BET762 might be useful tools to investigate the relationship between BET proteins and cytokine gene transcription [110, 132]. Disruption of BET protein binding to promoters in mouse bone marrow-derived macrophages results in suppression of 38 LPS-inducible genes, including *Il6*, *Ifnb1*, *Il1b*, *Il12a*, *Cxcl9*, and *Ccl12* [132]. Since this seminal finding, a growing body of evidence has supported a role for BET proteins in the regulation of inflammatory responses.

Of particular interest, BET proteins have been implicated in the regulation of Th17 inflammation. JQ1 inhibits retinoic acid receptor-related orphan receptor C (RORC—the Th17 master regulatory transcription factor) gene and protein expression in psoriatic mice [133]. By diminishing expression of RORC, BET inhibition reduces differentiation of Th17 cells as well as production of IL-17 and IL-22, which play a central role in Th17 inflammation as discussed above. BET proteins regulate the generation of Th17 cells and the

secretion of Th17 cytokines. The ability of BET inhibitors to block Th17 inflammation might make these inhibitors useful, in light of the emerging role of Th17 inflammation in T2D and breast cancer [18, 95, 103, 148, 149].

Several studies have more specifically explored the role of individual BET protein family member in regulation of transcription of cytokine genes. In primary epithelial cells, knockdown with siBRD2 had no effect on the secretion of IL-6, an effect which would be masked by pan-BET inhibitors [137]. Substantiating this role for BRD2, knockdown of BRD2 with siRNA inhibits LPS-induced secretion of pro-inflammatory cytokines from mouse bone marrow-derived macrophages. Although BRD4 is present, only BRD2 becomes enriched at the TNF- α promoter upon LPS stimulation [139]. For future research, it will be critical to consider the exclusive effects of individual BET family members when assessing BET protein regulation of cytokine networks.

BET inhibitors can also affect cytokine networks through indirect mechanisms. T-cell secretion of cytokines is dependent on stimuli from APCs, such as dendritic cells (DCs). JQ1 inhibits LPS-induced maturation of monocyte-derived dendritic cells (moDCs). JQ1-treated moDCs have a reduced ability to support Th1 differentiation, as well as CD4⁺ and CD8⁺ T cell proliferation and cytokine secretion in vitro [150]. Taken together, the current literature on BET protein regulation of inflammation provides a basis for further exploring epigenetic targets for the development of anti-inflammatory therapeutics.

BET inhibitors and clinical applications for inflammation

The discovery of inhibitors that are selective for BET protein bromodomains has not only advanced our knowledge of BET protein function, but has provoked a concerted effort to develop these inhibitors further [151], ultimately for clinical use. The first small molecules reported to disrupt BET bromodomain interactions with acetylated histones were I-BET and JQ1 [110, 132]. Past and recent development of BET bromodomain inhibitors has been focused on either increasing the affinity, specificity, and efficacy of current compounds, or on the development of new chemical families of inhibitors. Current approaches include optimizing BET bromodomain inhibitors based on the imidazo[1,2-a]pyrazine scaffold shared by both I-BET and JQ1, and synthesizing novel bromodomain inhibitors with dissociation constants in the nanomolar range [152, 153]. Efforts to develop novel BET inhibitors resulted in the development of the isoxazole azepine, the benzo[cd]indol-2(1H)-one, and the 5- and 6-isoxazolyl benzimidazole family of small molecules as “selective” inhibitors of the BET proteins [154–156], OTX015, and the Benzoisoxazoloazepine inhibitor (CPI-0610) [144]. Rvx-208 was designated as an inhibitor of BET proteins in 2013, although it was discovered in 2010 as a potent small molecule inducer of ApoA1 with an unknown mechanism [157]. This agent likely works by inhibiting BET proteins that normally act as co-repressors of transcription, thus their displacement from chromatin induces transcription [5].

Clinical use of pan-BET bromodomain inhibitors has focused on hematological cancers but has also shown efficacy for solid tumors [158–162]. In particular, BET proteins regulate multiple pro-tumorigenic pathways in breast cancer cells, such as drug resistance, cell

survival, proliferation, and invasion (reviewed in [162]). In vivo, JQ1 treatment significantly inhibits SUM1315 tumor growth in a xenograft mouse model [119]. Emerging data that support the efficacy of BET protein inhibition in solid tumors, including breast cancer, have provided a rationale for several clinical trials to test efficacy in humans [162].

Because BET proteins directly modulate cancer progression and inflammation, BET protein inhibition would essentially be a “two hit” approach for treating breast cancer in subjects with obesity-induced inflammation. In justification of this approach, the evidence base in support of BET inhibition for the treatment of inflammatory diseases is expanding. Complementing in vitro studies, preclinical and animal models indicate that BET inhibitors are effective for the treatment of inflammatory diseases in vivo (Table 1). Administration of JQ1 alleviates inflammation in mouse models of psoriasis, rheumatoid, and osteoarthritis (RA and OA), and multiple sclerosis (MS) [122, 127, 133, 135]. The ability of BET inhibition to treat these inflammatory diseases effectively in mice implies that BET inhibitors may be useful for the treatment of chronic inflammation associated with obesity, T2D, and breast cancers through epigenetic disruption of cytokine networks.

In light of the ubiquitous nature and diverse functions of the BET family of proteins, the need for BET inhibitors that can selectively target individual BET proteins is essential for safe transition of these inhibitors to the clinic. Currently, the potential side effects of using pan-BET bromodomain inhibitors are still under investigation. In animal models, JQ1 bioavailability is extremely high. After single-dose pharmacokinetic studies, it is evident that JQ1 is blood–brain barrier permeable ($AUC_{\text{brain}}/AUC_{\text{plasma}} = 98\%$) and also highly available in the testes ($AUC_{\text{testis}}/AUC_{\text{plasma}} = 259\%$) [163]. Indications that JQ1 treatment inhibits transcription in neurons and blocks memory in mice prompt concern regarding the use of pan-BET inhibitors in the clinic [164]. Furthermore, side effects of current BRD inhibitors remain significant. JQ1 reactivates HIV in patients with latent infections [165], causes uncontrolled increases in blood insulin [166, 167], and causes temporary male infertility due to inhibition of the testis-specific variant BRDT [163]. Bolden et al. demonstrated in mice that long-term suppression of BRD4 results in dramatic effects on multiple tissues, such as alopecia, stem cell depletion, and epidermal hyperplasia [168], which raises concerns over specificity. Therefore, safety concerns dictate that clinical use of BET proteins must be geared toward inhibitors that are selective among specific BET family members, coupled with tissue-specific drug targeting, to minimize the known side effects of BET protein inhibition.

Design of family member-selective inhibitors has implications for disease treatment. Because BET proteins share the structural bromodomains responsible for their epigenetic action, design of such selective inhibitors is difficult. Most efforts to develop selective inhibitors have been focused on BRD4 due to the discovery of a gene rearrangement that links BRD4 to aggressive carcinoma [169]. Additional efforts include the development of cell-based screening assays in BRD4-dependent cells [170], as well as designing small molecules that will target bromodomain 1 (BD1) of BRD4 [171, 172]. Taking the common approach to modify existing BET family inhibitors, I-BET and JQ1, Baud et al. developed an approach to introduce selectivity to BET bromodomain inhibitors [173], which involves Proteolysis Targeted Chimeras (PROTACs). These modifications tether JQ1 to a ligand for

the E3 ubiquitin ligase, which results in degradation of the BET protein [174]. Surprisingly, this approach results in selective degradation of BRD4 over BRD2 and BRD3, indicating the need for more structure/function analyses as we move toward the clinical exploitation of BET protein inhibition. An important evidence base shows that BRD2 plays a definitive role in regulating responses of immune cells [139, 166, 175]. Selective BRD2 inhibitors, designed in the context of anti-cancer treatments, would be advantageous to alleviate inflammatory diseases, while minimizing the potentially serious side effects of pan-BET inhibitors currently in clinical trials.

Conclusions

Chronic, unresolved inflammation offers one logical, functional link between the increased incidence and mortality of breast cancer in persons with obesity-associated co-morbid T2D. The ability of BET proteins to regulate inflammation makes them an attractive therapeutic target. Although not yet directly demonstrated in humans, T2D is likely accompanied by an inflammatory microenvironment in breast adipose tissue that could exacerbate breast cancer risk. Common inflammatory mediators implicated in T2D and breast cancer, which are also regulated by BET proteins, include IL-6, TNF- α , IL-1 β , and IL-17A [122, 132, 133]. The association of Th17 cytokines with both T2D and breast cancer, coupled with BET proteins' unique ability to regulate Th17 inflammation via multiple mechanisms, makes BET bromodomain inhibitors potentially strong candidates for the treatment of Th17 inflammation [18, 133].

Although many gaps remain in our understanding of the role of BET proteins in T2D-associated inflammation, one study has shed light on potential avenues of research to pursue. Unexpectedly, hypomorphic *Brd2^{wt/lo}* mice, which express low levels of Brd2 compared with wild type, become obese without metabolic disease, thereby uncoupling obesity from T2D [51]. Surprisingly, in these mice, macrophage infiltration into the abundant adipose tissue is greatly attenuated, suggesting a role for Brd2 in obesity-induced macrophage responses. Although the mechanisms underlying this phenomenon remain unclear, the ability of an epigenetic regulator to couple obesity to T2D and inflammation indicates that targeting cytokine networks through BET protein inhibitors may be a viable option. Future research should focus on the role of BET proteins in T2D inflammation, and whether BET-regulated cytokines in T2D affect breast cancer progression. Developing the links between obesity-associated T2D, breast cancer, and BET proteins may facilitate the use of BET proteins inhibitors for the treatment of chronic inflammation believed to drive these diseases.

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Fig. 1.

BET proteins regulate inflammatory processes implicated in obesity-driven type 2 diabetes and breast cancer progression. Several inflammatory processes are co-regulated by BET proteins. Some processes include cytokine gene transcription and transcription of NF-κB and STAT target genes [109, 112, 120, 129, 130]. The cytokines IL-17, IL-6, and IL-1β are implicated in both obesity-driven T2D and breast cancer, and are transcriptionally regulated by BET proteins [107–110]. Inflammation has been demonstrated to contribute to breast cancer aggressiveness and metastasis [5, 82–85]. Inflammation is also central to the pathogenesis of T2D, although the reciprocal relationship of T2D and inflammation is not completely understood [1, 24, 26–32]. ¹ T2D increases the risk of breast cancer incidence and mortality. ² Due to the role of inflammation in breast cancer, it is likely that diabetogenic inflammation contributes to the increase in breast cancer risk in T2D

Table 1

Summary of BET inhibitor anti-inflammatory effects in vitro and in vivo

BET Inhibitor	Inflammatory diseases ameliorated	Cytokines down regulated in vitro	Tissue types tested in vitro
JQ1 [110]	Psoriasis [133] and arthritis [122, 127, 135]	IL-1 β , TNF- α , IL-6, IL-8, and IL-10 [122, 136–138] [139]	Human airway smooth muscle cells [138], primary epithelial cells [137], multiple myeloma [136], RAW264.7 [126, 139], mouse macrophages [139], and fibroblast-like synoviocytes [122]
I-BET762 [132]	Neuroinflammation [140]	IL-1 β , IL-6, IL-10, IL-12, IL-17, IL-19, IL-23, IL-27, IL-33, and TNF- α , [132]	Human airway smooth muscle cells [138] and CD4 ⁺ T cells [140]
I-BET151 [141]	Multiple sclerosis [127]	IL-1 α , IL-1 β IL-6, IL-8, IFN- β , TNF- α , IL-23, and IFN- β [71, 125, 127]	Synovial fibroblasts [135] macrophages [125], and RAW264.7 [127]
OTX-015	N/A	IL-6 [142]	Primary human cell line -mantle cell lymphoma, multiple myeloma, splenic marginal zone lymphoma, and prolymphocytic leukemia [142]
CPI-203 [143]	N/A	N/A	N/A
CPI-0610 [144]	N/A	N/A	N/A
RVX-208 [145, 146]	N/A	N/A	N/A
LY294002 [147] ^a	N/A		

^aPI3 kinase inhibitor—not yet tested as a BET protein inhibitor targeted at inflammation