Review Article

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Crosstalk Involved In Bromodomain And Extraterminal Proteins Inhibition And Liver Diseases

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1. Abstract

Bromodomain and extraterminal (BET) proteins are a family of highly conserved proteins carrying a bromodomain (BD), which interbond with acetylated chromatin and as a key factor controlling transcription process, regulating a range of genes transcription. Previous studies have reported that BET proteins play an important role in the occurrence and progression of liver-related diseases, such as primary liver cancer and liver fibrosis. It is expected to be a new target for the treatment of liver-related diseases. Notability, BET proteins inhibitors have been proven to have tumor suppressive effects in some cancer models and considered as potential drug choices for cancer treatment. In this review, we focus on the mechanisms of BET proteins and its inhibitors involved in the progression and treatment of various liver-related diseases from liver fibrosis to liver cancer. At the same time, we discuss the challenges and future directions of BET proteins in liver health and diseases.

2. Keywords:

BET Proteins, Histone Acetylation, Liver-Related Diseases, BET Proteins Inhibitors

3. Abbreviations:

BET: bromodomain and extraterminal, BD: bromodomain, ET: extraterminal, eRNAs: enhancer RNAs, HCC: hepatocellular carcinoma, PES1: pescadillo homolog 1, NASH: nonalcoholic steatohepatitis, miRNA: microRNA, EGFR: epidermal growth factor receptor, MDSCs: myeloid-derived suppressor cells, ICBs: immune checkpoint blockers, VEGF: vascular endothelial growth factor, EMT: epithelial-mesenchymal transition, SPZ1: spermatogenic leucine zipper 1, NAFLD: nonalcoholic steatohepatitis, HSCs: hepatic stellate cells, EZH2: enhancer of zeste homolog 2, TLR2: Toll-like receptor 2, SREBP1c: sterol regulatory element binding protein 1c, BECs: biliary epithelial cells, HB-LCs: hepatoblast-like cells, HDACII: histone deacetylase II, NREs: nodal gene regulatory elements.

4. Introduction

The dynamic regulation of histone acetylation affects chromatin structure, DNA accessibility and transcriptional activity [1]. Numerous studies have reported the roles of histone acetylation (such as that mediated by histone acetyltransferase) and deacetylation (for example, histone deacetylases) in liver diseases [2-4]. In recent years, the function of histone acetylation readers in liver-related diseases has become a research hotspot. Following the discovery of small-molecule inhibitors of bromodomain and extraterminal (BET) proteins, the potential therapeutic effects targeting members of the BET proteins family in preclinical research on a range of diseases, including cancer, autoimmune disorders, neurodegenerative diseases, and endocrine- and metabolic-related disorders, have attracted increasing attention [5-7]. In this review, we focus on BET proteins as histone acetylation reader, summarizing their structure and novel selective pharmacological inhibition. We highlight the regulatory mechanisms by which BET proteins and their inhibitors affect the progression and treatment of various liver-related diseases, from liver fibrosis to liver cancer. Finally, we discuss the challenges and future directions of BET proteins in liver health and disease.

5. Structures Of BET Proteins

BET proteins constitute a family of highly conserved proteins carrying a bromodomain (BD). In mammalian cells, the BET proteins family consists of four members: BRD2, BRD3, BRD4 and BRDT. Among these proteins, BRD2, BRD3 and BRD4 are expressed in most cells and tissues,

while BRDT is expressed mainly in the testis [8,9]. In BET proteins, there are two BDs (BD1 and BD2) comprising approximately 110 amino acids and an extraterminal domain (ET), with BRD4 and BRDT also harboring a carboxy-terminal motif (CTM) [10].

Two tandem BDs (BD1 and BD2) in BET proteins recognize and bind to the lysine residues of acetylated histones (e.g., H3K27ac, H4K5ac, and H4K12ac) or acetylated nonhistone proteins (NF-κB, Twist, and the RelA subunits of GATA1) [11-14]. Lysine residue (Kac site) acetylation is an important posttranslational modification of histones. When histones are acetylated, their nucleosomes become less stable and more compact, resulting in altered or blocked protein–protein or protein–DNA interactions [15, 16]. Histone acetylation is a major contributor to the dynamic regulation of gene expression by histone modification complexes and is considered a marker of open chromatin, allowing not only transcription factors but also components of DNA polymerase or DNA repair mechanisms to access DNA [17]. Therefore, BET proteins plays a very important regulatory role in gene transcription. In addition, the affinity of BET proteins for specific acetylated histones is largely affected by the modification of adjacent histones, which indicates that BET proteins are more likely to recognize a combination of modifications than any single acetylation modification site [12]. The amino acid sequences of BD1 and BD2 are clearly different, which is the basis for the selective inhibition of BD1 and BD2[18].

In addition to a BD, BET proteins carry an extraterminal domain, the interaction of ET and chromatin involves adjustment factor (for example, NSD3, JMJD6 and CHD4) as well as frequent interactions between viruses and gene loci [19-21]. In addition, the BRD4- and BRDT-specific CTMs play important roles in regulating the activity of transcription elongation factor b (P-TEFb) and the transcription elongation process [22-24]. Thus, by linking acetylated histones to transcription complexes, BET proteins plays a unique role in regulating the expression of related genes.

6. Functions Of BET Proteins

In the BET proteins family, BRD2 is primarily involved in the transcription of genes that regulate embryonic development, neurodevelopment, and cell cycle activity in cells after cancers/tumors have developed [25,26]. The known function of BRD3 is primarily associated with regulating the transcription of genes required for embryonic stem cell development [27,28]. BRDT expression is limited to the testes, and BRDT knockout or mutation in mice results in a reduced sperm count and abnormal sperm morphology, indicating its role in spermatogenesis [29,30].

Compared with other members of the protein family, BRD4 has attracted more attention. Although BRD2, BRD3, and BRD4 share similar domains (e.g., BD1, BD2, and the ET), many studies on protein interactions and mechanisms have focused on BRD4. Initially, most studies showed that the function of BRD4 in regulating gene transcription is targeted mainly to promoter regions, but recent studies have shown that the gene-regulating effect of BRD4 is also related to BRD4 occupation in intergenic regions and gene bodies, not merely at a transcription start site[31]. The interaction of BRD4 with enhancers and superenhancers at genomic regions has been another hot topic in recent research. Together with a mediator complex, enhancers associated with BRD4 binding can activate P-TEFb on the promoter region through long-distance enhancer–promoter interactions [32]. In addition, the BD in BRD4 interacts with P300/CBP to increase the acetylation rate at histone H3K27, and this modification is typically enriched in enhancer and superenhancer regions[33]. The binding of BRD4 to enhancer regions can modulate the expression of enhancer RNAs (eRNAs), thereby affecting the activity of BRD4[34]. For example, a recent study confirmed that eRNA enhanced the binding of BD to histone H3K27ac and H4K16ac sites in BRD4 to regulate gene expression. Given that most eRNAs are expressed in a cell type-specific manner, further research is needed to determine whether BET inhibitors produce cell type-specific effects in the liver by disrupting BRD4 enhancers and/or BRD4–eRNA interactions.

To date, although the biological function of BRD4 is clearly understood, the specific mechanism by which BRD4 activates, and recruit's chromatin remains unclear. It has been proposed that BRD4 is regulated via a "phosphorylation switch" mechanism. This mechanism is thought to affect the ability of BRD4 to bind chromatin and interact with acetylated proteins[35]. Proteins such as casein kinase II and protein kinase A phosphorylate the N-terminal phosphorylation site of BRD4, and protein phosphatase 2 removes phosphate groups from these sites. When BRD4 is dephosphorylated, the binding of the BD in BRD4 to acetylated regions of a histone or nonhistone protein is inhibited, and when BRD4 is phosphorylated, the BD interaction with acetylated proteins is increased[36]. Therefore, the detection and regulation of the phosphorylation status of BRD4 may be necessary to determine its functional status under specific disease conditions. In addition to functioning as a reader for acetylated lysine residues and a scaffold for various chromatin modification factors and transcription factors, BRD4 may also exhibit kinase and histone acetyltransferase activities. As an atypical kinase, BRD4 promotes transcription by specifically phosphorylating a serine residue in the carboxy-terminal domain of RNA polymerase II[37]. BRD4 also function as a histone acetyltransferase, acetylating the spherical region of histone H3K122 residues, resulting in increased chromatin accessibility[38]. These new findings add to the appreciation of the complexity of BRD4 function.

7. Inhibitors Of BET Proteins

In 2010, researchers developed two small-molecule inhibitors based on the structure of benzotriazolodiazepine (called I-BET) and thienotriazolodiazepine (called JQ1), which can selectively inhibit the binding of BD in BET proteins to acetylated histones [39,40]. Although JQ1, I-BET151 and similar BET proteins inhibitors show higher specificity for the BD in BET proteins than the BD in non-BET proteins, these small-molecule inhibitors can bind to the BD1 and BD2 domains

of any BET proteins and cannot selectively distinguish BRD2, BRD3, BRD4 and BRDT. In recent years, c have been continuously improved in terms of selectivity, effectiveness and pharmacokinetics, and more than a dozen small-molecule drugs have been developed and mainly used for preclinical research evaluation of cancer and other related diseases[41]. Recently, BET proteins inhibitors that selectively target BD1 (GSK778, MS-436, Olinone, and BI-2536) and BET proteins inhibitors that selectively target BD2 (GSK046, RVX-208, RVX-297, ABBV-744) have been developed [42-47]. In contrast to pan-BET proteins inhibitors, these selective BET proteins inhibitors of BD1 or BD2 are characterized by discrete transcriptional profiles in vitro [48]. A cancer model study showed that the BET inhibitor GSK778 targeting BD1 showed regulatory effects on transcription similar to that of the pan-BET inhibitor, while the BET inhibitor GSK046 targeting BD2 exerted a different regulatory effect on transcription production, showing limited inhibitory effects on BRD4 binding to chromatin and cell proliferation. However, it led to a more effective response as a treatment in studies with models related to inflammatory and autoimmune diseases. In contrast to pan-BET proteins inhibitors, highly selective protein inhibitors of BD2 mainly modify previously induced gene transcription while the established transcription program is largely unchanged[42].

Recently, reported drug screening experiments have shown that certain kinase inhibitors can also function as effective BET proteins inhibitors. For example, dinaciclib (CDK inhibitor), TG101209 (JAK2 inhibitor), and BI-2536 (PLK1 inhibitor) inhibit BET proteins function at submicromolar concentrations[49,50]. Therefore, the effects of using these kinase inhibitors observed in previous studies may be partially attributable to their inhibitory effect on functions of BET proteins. These findings have led to the design and development of dual inhibitors of multiple BET proteins and other kinases[51]. For example, these inhibitors target BET proteins and a histone deacetylase, BET proteins and a histone acetyltransferase, among other dual inhibitors [52,53]. Although these small-molecule compounds have been studied primarily in cancer models, further research is needed to determine whether dual-function BET proteins inhibitors can be used for the treatment of liver-related diseases.

The aforementioned small-molecule drugs interfere with the interaction of the BD in BET proteins with acetylated histone and nonhistone proteins. Since BET proteins also function independent of their BDs, new strategies have recently been developed to investigate other molecules that inhibit functions of BET proteins. For example, in a technique called proteolytic targeting chimera (PROTAC), which pairs BET inhibitors (e.g., JQ1) with a ubiquitin ligase recognition module[54], JQ1 competitively displaces acetylated chromatin to bind to BET proteins. Moreover, a E3 ubiquitin ligase was recruited to the ubiquitin ligase recognition site, which led to the ubiquitination and proteasome-dependent degradation of the targeted BET proteins. PROTAC compounds (such as dBET1, ARV825, and MZ1) provide novel ways to inhibit the overall function of BET proteins[55-57]. In addition, blocking the interaction between phosphorylated BRD4 and transcription factors is another novel therapeutic strategy to regulate the function of BRD4 without inhibiting the activity of the relevant domain in BRD4. Recently, cell-permeable peptides (DC-1 and DC-2) were shown to bind specifically to the phosphorylated N-terminal site of BRD4 and thus block BRD4 phosphorylation, specifically inhibiting the general function of BRD4 [58]. With an increasing number of small -molecule drugs with high selectivity and versatility available to regulate the activity of BET proteins, the prospects for exploring the therapeutic effects of BET proteins inhibitors on liver-related diseases are expanding.

In addition, the tolerance and side effects of BET inhibitors are unavoidable problems. The main side effects discovered through research and related to BET inhibitors are thrombocytopenia and gastrointestinal toxicity[59, 60]. These side effects are significantly dose dependent and are reversed after the discontinuation or elimination of BET inhibitors. However, to date, no clinical studies using BET inhibitors have been used to treat liver disease; therefore, the side effects of BET inhibitors associated with the treatment of liver-related diseases remain a major unknown. Utilizing BET proteins inhibitors with relatively short half-lives may be the preferred approach to normalize disease-associated transcriptional responses while limiting nonspecific side effects. Determining whether more selective inhibition of BET proteins activity exerts similar therapeutic effects and fewer side effects is one of the goals of preclinical and clinical studies. Small-molecule compounds that selectively target BD1 and BD2 in BET proteins have been developed, and satisfactory results have been obtained in preliminary clinical studies[45, 61,62]. Other methods to inhibit BET proteins function independent of the BD have also recently been reported [54, 63], but their therapeutic effect in liver-related disease models has yet to be verified.

8. Inhibition Of BET Proteins Function And Treatment Of Liver Cancer

BET proteins, as an epigenetic regulator of the cell cycle, proliferation, and apoptosis, has become a potential target in cancer therapy. Some previous studies have shown that BET proteins plays an important role in the occurrence and progression of hepatocellular carcinoma (HCC). HCC is the main type of primary liver cancer. Previous studies have found that BRD4 is overexpressed in HCC cells compared with normal liver cells in both in vivo and in vitro experiments. Silent expression of BRD4 or the use of specific inhibitors can inhibit the proliferation, migration and invasion of HCC cells and induce apoptosis of HCC cells. It also inhibited the growth of xenografted mouse HCC tumors [64-66].

How BET proteins inhibitors regulate cell proliferation and apoptosis and the identification of the mechanisms underlying these effects are hot research topics; these mechanisms clearly involve multiple signaling pathways and targets, but no consensus on their identifies has been reached present date. Studies have confirmed that after JQ1 treatment of HCC cells in vitro, inhibition of the BRD4-c-Myc-p27 signaling axis causes the cell cycle of HCC cells to stagnate in the G1 phase, and thus, apoptosis is induced[66]. Another study revealed that inhibiting

BRD4 led to changes in gene expression independent of c-Myc pathway activation in HCC cells. A gene expression analysis of JQ1-treated HCC cells showed that E2F2 was a direct downstream target of BRD4. Further studies have confirmed that E2F2 is a key factor in the regulation of HCC progression mediated by BRD4 inhibitors, and high expression of E2F2 is significantly correlated with poor prognosis in HCC patients [67].

Other studies have confirmed that the BET proteins inhibitor BETd-260 effectively inhibits cell proliferation and induces apoptosis in HCC cells. BETd-260 regulates the expression of apoptosis-related genes in HCC cells; that is, it inhibits the expression of antiapoptosis-related genes such as Mcl-1, Bcl-2, c-Myc and X-linked apoptosis inhibitors and increases the expression of the pro-apoptotic gene Bad. BETd-260 treatment leads to the destruction of mitochondrial membrane integrity in HCC cells and triggers intrinsic apoptosis signaling pathways in HCC cells[68]. Another study analyzed the effect of JQ1 on the gene expression of members in the Bcl-2 family by RNA sequencing and found that JQ1 triggered the upregulation of Mcl-1 in HCC cells. Silencing the expression of Mcl-1 significantly enhanced JQ1-induced caspase-3 activation, cleavage of ADP-ribose polymerase, and induction of apoptosis in various HCC cell lines[69]. In addition, BRD4 exerted a regulatory effect on the expression of Pescadillo homolog 1 (PES1), which is involved in rRNA processing and ribosome synthesis, resulting in antitumor effects. Previous studies have confirmed that improper recruitment of PES1 leads to the occurrence of various types of cancer, and the upregulation of PES1 expression promotes the proliferation of HCC cells and is associated with poor prognosis in HCC patients. Inhibition of PES1 expression reduces the glycolysis rate by altering the gene expression of GLUT1, PKM2, ENO1, FBP1, and PCK1, which are associated with glucose metabolism in HCC cells[73].

In summary, although a consensus has been reached indicating that inhibition of BET proteins expression or the use of BET proteins inhibitors inhibits HCC cell proliferation and induces apoptosis, the explanation of the mechanisms is inconsistent and thus remain unclear. Therefore, these mechanisms need to be further studied in the future. In addition, some recent studies have established a causal relationship between epigenetic changes and hepatocellular carcinoma, suggesting that therapies targeting epigenetic modifications may show potential for tumor therapy, which involves a role for the targeted inhibition of BET proteins in HCC therapy. One study examined the clinical relevance of epigenetic modifiers in HCC, including BDs, histone acetyltransferases, lysine methyltransferases, and lysine demethylases, by analyzing genomic mapping data from cancer tissues of 365 HCC patients. The correlation between the alteration, abnormal expression, and expression of epigenetic modification enzyme-encoding genes in tumors and poor HCC prognosis was found to be a common event in HCC. Inhibitors targeting epigenetic modification enzymes such as the BD (JQ1), a lysine methyltransferase (BIX-1294 and LLY-507), and a lysine demethylase (JIB-04, GSK-J4, and SD-70) reduced HCC aggressiveness [71]. Another study revealed that chronic viral hepatitis and nonalcoholic steatohepatitis (NASH) are characterized by similar epigenetic and transcriptional modifications that contribute to the development of HCC, increasing the risk of cancer. By analyzing epigenetic modifications in clinical patient samples, researchers identified chromatin readers as key factors in regulating gene transcription in the liver and that drives the development of HCC. In mouse NASH-HCC models, BET proteins inhibitors inhibited the progression of liver disease and the occurrence of liver cancer by restoring the transcriptional reprogramming of epigenetically altered genes[72].

In recent years, increasing evidence has shown that abnormal microRNA (miRNA) expression is related to the progression of HCC, and BET proteins play an important regulatory role in this process. The results of one study showed that the expression of miR-329 was significantly reduced in HCC samples from patients, and the low expression of miR-329 promoted tumor progression. By targeting BRD4, miR-329 regulates the invasion ability of cells but exerts no effect on the proliferation and apoptosis of HCC cells [73]. Another study confirmed that upregulation of BRD4 expression in human HCC tissues was associated with downregulation of miR-608 expression. BRD4 is the main target gene of miR-608 in HCC cells. After silencing BRD4 expression in HepG2 cells, the Mir-608-induced downregulation of c-Myc and inhibition of cell proliferation in HepG2 cells were prevented. In vivo experiments showed that overexpression of miR-608 or silencing of BRD4 expression significantly inhibited the growth of HepG2 xenograft tumors[74].

Although previous studies have shown the effectiveness of BET proteins inhibitors in the treatment of cancer, other studies have revealed that certain tumor cells develop resistance to BET inhibitors, greatly limiting their therapeutic potential. Because c-Myc is frequently overexpressed in HCC tissues and cells, targeted inhibition of c-Myc and its associated signaling pathways may be critical for the treatment of advanced HCC. The BET proteins inhibitor JQ1 was used to treat HCC cells with high c-Myc expression, and the results showed a stronger antitumor response than that obtained with sorafenib, and the JQ1 treatment exerted significantly greater damaged on mitochondrial respiration and glycolysis in the HCC cells. However, in some HCC patients, with high expression of both c-Myc and epidermal growth factor receptor (EGFR), the antitumor effect of JQ1 was unsatisfactory. Studies confirmed that by inhibiting the signaling axis of EGFR-activated protein kinase (MAPK), JQ1 further enhances the effectiveness of the HCC antitumor response by stabilizing the expression of the c-Myc gene. Therefore, inhibition of EGFR activity significantly reduces the expression level of c-Myc, greatly reduces drug resistance to JQ1 and enhances the antitumor effect of JQ1[75].

In recent years, the research progress of combined targeted therapy of tumors has been very rapid and is expected to become a new form of HCC treatment. BET proteins inhibitors combined with other targeted drugs compensate for the shortcomings of the other in the treatment of HCC. Due to the relatively low responsiveness of HCC to immune checkpoint blockers (ICBs), the strategy of BET proteins inhibitors combined with ICBs has received increasing attention in recent years,

but the mechanisms of action diverge. Myeloid-derived suppressor cells (MDSCs) are essential for immunosuppression, monocytic MDSCs (M-MDSCs) in model mice with fibrosis and HCC were found in the fibrotic liver. However, an accumulation of polymorphonuclear MDSCs (PMN-MDSCs) was significantly associated with a decrease in the number of tumor-infiltrating lymphocytes (TILs) and tumor progression. A combination treatment consisting of the BET proteins inhibitor i-BET762 and an anti-PD-L1 antibody inhibited the accumulation of M-MDSCs and synergistically enhanced the number and function of TILs, thereby inhibiting HCC progression and prolonging survival in model mice with fibrosis and HCC [76]. Our previous study revealed that JQ1 monotherapy was not effective in inhibiting HCC progression in model mice with primary HCC. This mechanistic study showed that although JQ1 treatment did not affect the total expression level of PD-L1 in cells, it upregulated the expression of PD-L1 on the cell membrane, which was mediated by JQ1, which regulated the expression of Rab8A. We confirmed that the therapeutic strategy of combining JQ1 with an anti-PD-L1 antibody led to effective inhibition of HCC progression, and the number of CD8-positive T cells among immune cells infiltrating the liver increased significantly after administration of the combination treatment. In addition, the activation and release rates of cytotoxic factors were significantly enhanced. Other studies confirmed that Mcl-1 is the main contributing factor of BET inhibitor resistance in HCC cells. JQ1 treatment in combination with drugs that downregulate Mcl-1, such as the cyclin-dependent kinase inhibitor flavopiridol, significantly inhibited the expression of Mcl-1. The result was rapid apoptosis of the HCCLM3 and BEL7402 cells lines, suggesting that the combination shows an increased therapeutic effect on HCC cells [69].

In addition to HCC, BET proteins inhibitors have been studied for use in the treatment of other types of liver cancer. In a study of liver metastatic carcinoma, spermatogenic leucine zipper 1 (SPZ1) was found to function as a proto-oncogene and promote the epithelial-mesenchymal transition (EMT) during tumor development. After acetylation of the SPZ1-TWIST1 complex, Vascular endothelial growth factor (VEGF) expression was enhanced by BRD4, RNA-Pol II-dependent transcription was enhanced, and tumor metastasis was induced. Neutralizing VEGF with humanized monoclonal antibodies, such as Avastin, effectively abrogated the EMT and tumor metastasis induced by the acetylation of the SPZ1-TWIST1 complex. This study confirmed the importance of acetylation signaling in the SPZ1-TWIST1-BRD4 axis in the regulation of EMT-mediated tumor initiation and metastasis [77]. In addition, other studies have confirmed that BRD4 expression in cholangiocarcinoma tissues was significantly higher than that in normal bile duct or surrounding normal liver tissue. Silencing the expression of BRD4 in bile duct cancer cells inhibited the expression level of c-Myc, upregulated the expression of p21, stalled the cell cycle in the G1 phase, inhibited the proliferation of bile duct cancer cells and induced apoptosis [78]. In conclusion, the therapeutic effects of BET proteins inhibitors in other types of liver cancer are relatively limited, and further study is needed.

9. The Inhibition Of BET Proteins Function And Treatment Of Liver Fibrosis

In the liver, various etiologies include chronic viral hepatitis, nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD)/NASH, obesity/metabolic syndrome and inflammation. Chronic liver injury leads to the formation of liver fibrosis. Liver fibrosis is a result of dysregulated healing processes after liver damage and is characterized by excessive deposition of extracellular matrix. Activated in response to liver injury, hepatic stellate cells (HSCs) are the primary sources of extracellular matrix and drive the healing process after liver injury. However, some chronic liver injuries lead to the permanent activation of HSCs and the progressive formation of pathological scar tissue, ultimately leading to liver fibrosis and liver failure [79]. Although great progress has been made in the study of liver fibrosis, some mechanisms of liver fibrosis are still unclear. To date, no effective therapy has been found for the early and reversible stage of liver fibrosis. Although the fibrosis caused by chronic viral hepatitis can be treated with antiviral therapy, liver fibrosis caused by nonviral etiology is still difficult to treat[80,81], and once it progresses, treatment is limited to slowing the progression of the disease and cannot reverse liver fibrosis[82]. Although the advanced stage of liver fibrosis is irreversible, recent animal models and clinical studies have confirmed that liver fibrosis and even the early stage of cirrhosis are still reversible[83].

In recent years, with the study of the mechanism underlying BET proteins and its inhibitors, BET has been shown to play an important role in the progression and treatment of liver fibrosis. In mouse models of NAFLD/NASH, the BET proteins inhibitor I-BET151 reduced the NAFLD activity score, which is a clinical measure to assess the severity of NAFLD as well as the progression of liver fibrosis and interferon-gamma expression. RNA sequencing confirmed that I-BET151 treatment changes the molecular signaling pathways related to interferon signaling and cholesterol biosynthesis and can reverse the expression of signature genes associated with liver fibrosis, suggesting that BET proteins may be key targets in the treatment of NASH and liver fibrosis [84].

Cholestasis causes persistent liver damage and may eventually lead to liver fibrosis. In an animal model of liver fibrosis induced by bile duct ligation (BDL), miRNA-29a (miR-29a) can further regulate the development of cholestatic liver fibrosis by targeting BRD4. miR-29a negatively regulates the activation of HSCs by inhibiting the gene expression of Enhancer of zeste homolog 2 (EZH2), zeste, and BRD4, and alleviate liver fibrosis [85]. Another study also confirmed that in the BDL model comprising miR-29a transgenic (miR-29a Tg) mice, the liver tissue showed less fibrosis than that of wild-type mice. Overexpression of miR-29a in HSCs downregulated the expression of EZH2, MeCP2 and SNAI1 and upregulated the expression of PPAR-γ. The BRD4 inhibitor JQ1 inhibited the expression of C-MYC, EZH2 and SNAI1 in HSCs, while both miR-29a and JQ1 inhibited the activity and proliferation of HSCs. In addition, Toll-like receptor 2 (TLR2) and TLR4, which are pattern recognition receptors for bacterial lipoproteins and lipopolysaccharides, are also involved in the activation of HSCs and liver fibrosis. Studies have confirmed that miR-29a

also inhibited the TLR2 and TLR4 signaling pathways, thereby slowing liver fibrosis caused by cholestasis[86], but whether BRD4 also plays a key regulatory role in this process is unclear, and further research is needed. The activation of HSCs is largely triggered by mechanical signaling in the microenvironment, which induces and promotes the activation of HSCs and fibrotic transcription programs related to extracellular matrix secretion, thereby establishing a positive feedback loop that leads to stromal scarring and self-sustaining pathological HSC activation. Sterol regulatory element binding protein 1c (SREBP1c) plays a key role in the regulation of HSC activity. Some studies found that SREBP1c inhibited the activation of HSCs by downregulating the expression of BRD4 in HSCs and exerted a significant inhibitory effect on thioacetamide-induced liver fibrosis. The potential mechanism underlying the effect of SREBP1c on HSC activation and liver fibrosis involves its regulatory effects on downstream signaling and the expression of epigenetic modification-regulating BET proteins[87].

Collagen activates fibrosis factors in a matrix-dependent manner; for example, it increases α-SMA expression and promotes TGF-β activation by applying force to collagen-binding integrin receptors, leading to downstream mechanical conduction and resulting in the formation of stress fibers[88]. Inhibition of αvβ1 integrin inhibited TGF-β activation and alleviated bleomycin-induced lung and CCL4-induced liver fibrosis in mice [89]. Thus, collagen deposition leading to integrin activation and mechanical transduction establishes a feedback loop that promotes pathological extracellular matrix deposition and a sustained fibrosis in response. A study on renal fibrosis confirmed that inhibition of BRD4 activity prevented the formation of renal fibers by blocking the TGF-β-NOX4-ROS-fibrosis signaling axis [90]. Considering that BRD4 regulates TGF-β-mediated collagen deposition during fibrosis, a compelling mechanistic hypothesis suggests that BRD4 enhances signaling through integrin-mediated mechanotransduction pathways, thereby exacerbating TGF-β-mediated liver fibrosis, and leading to pathological extracellular matrix remodeling. This remodeling would lead to increased tissue hardness and rigidity and sustained HSC activation.

10. BET Proteins Function Inhibition And Liver Regeneration

The liver shows extraordinary regenerative capacity. In ancient Greek mythology, Prometheus stole fire, so Zeus allowed the fire god put Prometheus on a cliff and to send an eagle to peck at his liver; his liver was eaten every night, and the pain was endless [91]. The effectiveness of treatment for many diseases, such as liver surgery (tumor resection and donor liver transplantation), liver trauma or liver poisoning, depends on effective liver regeneration [92,93]; therefore, the liver regeneration process is of great clinical significance. During liver regeneration, liver cells are mainly derived from preexisting liver cells but can also originate from other types of cells in the liver. For example, when hepatocyte proliferation is extensively impaired, biliary epithelial cells (BECs) be sources of new hepatocytes. In the past few decades, many studies have modeled the liver regeneration process using partial liver resection and toxin-induced liver injury in rodents and investigated the important role of various cell populations and signaling pathways in regulating the liver regeneration process[92, 93]. Compared with the extensive research on BET proteins in cancer, the role of BET proteins in liver regeneration or liver biology has been reported rarely [94,95].

Using a mouse model of 70% partial hepatectomy (PHx), we confirmed in previous studies that JQ1 therapy significantly inhibits liver regeneration at an early stage. Mechanistic studies have revealed that BET proteins play key regulatory roles in hepatocellular-driven liver regeneration through the YAP/TAZ-NOTCH1-NICD axis. Another study reported damage to liver regeneration by BET proteins inhibitors in a mouse model after 70% PHx and in zebrafish liver with injury induced by acetaminophen. Treatment with JQ1 after 70% PHx resulted in increased liver damage and almost complete inhibition of hepatocyte proliferation. In a zebrafish liver injury model induced by acetaminophen, JQ1 treatment showed an obvious inhibitory effect on hepatocyte proliferation. In both models, the Wnt signaling pathway was significantly inhibited by JQ1. This study revealed that the BET proteins can regulate the liver regeneration process driven by hepatocyte proliferation through the Wnt signaling pathway[96]. In addition, another study demonstrated that in addition to liver cells, BET proteins can regulate the BEC-driven liver regeneration process. The process of liver regeneration driven by BECs includes BEC dedifferentiation into hepatoblast-like cells (HB-LCs), which further proliferate and differentiate into new normal hepatocytes[97]. In a zebrafish liver regeneration model, BET proteins inhibitors JQ1 and iBET151 inhibited the expression of HB-LC signature genes Prox1 and Hnf4a in BECs. After hepatocyte ablation, BET inhibitors prevented BECs from dedifferentiating into HB-LCs. Liver regeneration was resumed after JQ1 was eluted, suggesting that BET inhibition temporarily but not permanently interferes with liver regeneration. BET inhibitors administered after hepatocyte ablation inhibited the proliferation of newly generated hepatocytes and delayed the maturation of hepatocytes [98].

11. BET Proteins Function Inhibition And Other Liver Diseases

In addition, BET proteins also play important regulatory roles in liver lipid metabolism. Histone deacetylase II (HDACII) plays an important role in metabolic regulation. HDACII-deficient mice exhibit enhanced thermogenic potential, reduced obesity, increased insulin sensitivity, and reduced hepatic steatosis in response to feeding with a high-fat diet. Studies have shown that BRD2 catalyzed the activity of HDACII by binding to it and regulated the transcriptional program of proteins related to liver lipid metabolism in the basal state and in response to β-adrenergic receptor signaling[99]. In addition, excessive fructose intake induced hepatic steatosis by activating fat synthesis. Mechanistic studies have revealed that this outcome is related to the upregulation of lipid accumulation genes (such as Cyp8b1, Dak, and Plin5) after fructose intake. Mandatory fructose intake enhanced acetylation of histones H3 and H4 and BRD4 binding to regions associated with lipid storage gene transcription, and JQ1 treatment reduced the expression of fructose-induced lipid storage-

associated genes [100]. These findings suggest that epigenetic pathways regulate energy homeostasis and hint at a potential role for BET proteins inhibitors in the treatment of obesity and diabetes. Liver pluripotent stem cells (PSCs) show great clinical potential because of their ability to differentiate into fully specialized tissues. Recent studies have shown that BET proteins bind specifically to Nodal gene regulatory elements (NREs) to promote Nodal signaling and the Smad2 developmental response, and the Smad2 signaling network is a key determinant of PSC fate. In pluripotent cells, both BRD2 and BRD4 bind to NRE, and the decreased rate of BRD4 binding to NREs and the increased rate of BRD2 binding to NREs attenuate hepatic PSC pluripotency. Thus, BRD4-BRD2 isotransformation at NREs coordinates the attenuation of hepatic PSC pluripotency[101].

12. Summary And Future Directions

Because BET proteins play key roles in regulating transcriptional responses, BET proteins has become a hot subject in the field of liver health and disease. Although much progress has been made in treatments with BET proteins, many questions remain unanswered. For example, most research has focused on BRD4, and the roles that BRD2 and BRD3 play in liver-related diseases are unclear. Because the functions of BET proteins family members do not completely overlap, each shows certain unique activity [102] and BET proteins inhibitors developed to date are not selective for BRD4, more studies are needed to clarify the mechanism of action underlying the effects of BET proteins family members on liver-related diseases. Further understanding of signaling upstream of BET proteins in the liver (e.g., receptor signaling cascades, posttranslational modifications to individual BET proteins), downstream target molecules (e.g., histones, nonhistones, eRNAs, and genome-binding regions), and cell type-specific mechanisms assessed in a single type of medium is needed for realizing BET proteins-targeted treatment of liver-related diseases.

Finally, for many noncancerous liver-related diseases, the effective uses of BET proteins inhibitors for treatment remain unclear. For example, can BET proteins inhibitors be combined with FDA-approved drugs to produce a synergistic effect? Do BET proteins inhibitors require specific packaging treatments (e.g., nanoparticles) to be effectively absorbed by the liver or specific cell populations within the liver? Ongoing efforts to address these questions reveal the role played by BET proteins in liver-related health and disease.

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