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Novel approaches to targeting BRD4

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Inhibition of bromo and extra-terminal (BET) bromodomains, including BRD4, has emerged as a new exciting epigenetic target for oncology, in particular. Recently, novel alternatives to the traditional use of reversible small molecules have emerged, including proteolytic targeting BET agents and irreversible binding inhibitors. These alternatives to reversible inhibitors may offer some advantage and can be used as tools to further decipher the underlying biology. Supportive pre-clinical data have these novel approaches bound for clinical development in the near future.

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Introduction

Protein silencing or degradation has proven to be a valuable part of the functional proteomics toolbox, allowing researchers to control intracellular protein levels. It complements other valuable tools such as the use of knockout mice and RNAi knockdowns, which are applied at the gene and transcriptional levels, respectively. Protein silencing has been used to study the function of proteins [1] and target validation [2]. Recently, protein silencing or degradation has been applied to the development of inhibitors of bromo and extra-terminal (BET) bromodomain BRD4. The inhibition of BRD4, in particular, has been described as a promising strategy to disrupt the transcriptional programs associated with the proliferation and progression in several cancer types [3]. In this review, we discuss recent advancements in targeting of BRD4 using protein silencing tools and irreversible inhibitors, focusing on their potential as drug candidates.

Current status of BET inhibitor development

Small-molecule BET inhibitors have recently emerged as an attractive therapeutic approach to target BET bromodomains, which have been described to be involved in various disease indications, including hematological and solid tumor cancers as well as autoimmune and cardiovascular diseases [4]. In their mechanism of action, BET inhibitors bind to the bromodomains of the BET family proteins, consisting of BRD2/3/4 and testis-specific BRDT, and disrupt their interaction with the acetyl lysine residues of histones and transcriptional factors, leading to alterations of transcription and gene expression, which further impact several disease states [4]. Each BET protein contains a tandem bromodomain module (BD1 and BD2) to which BET inhibitors can bind with an equal affinity or with preferential binding selectivity to one of the two domains. BRD4, in particular, is an attractive therapeutic target due to its well-established role in cancer, inflammation, and other diseases [4,5].

The expanding field related to the understanding of BET inhibitors continues to advance, looking for effective and targeted treatments in several disease areas [6]. Some of these small-molecule inhibitors have now entered clinical development, mainly in oncology [3,6–8]. Substantial clinical data from these new inhibitors are expected over the next few years.

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Although the clinical development of BET inhibitors is at an early stage, some preliminary safety data have emerged. One of the BET inhibitors, OTX-015 (now MK-8628), a thienotriazolodiazepine, is undergoing Phase 1 clinical trials in hematological and solid tumor cancers [9–11]. In addition to some evidence of clinical efficacy, dosing with OTX-015 also has resulted in effects on platelets, primarily thrombocytopenia, and some gastrointestinal events [12]. Also, gastrointestinal toxicity has been reported from pre-clinical work [13]. In rats, BET inhibition has been shown to result in loss of fast-cycling intestinal stem cells [14]. Also, complete knockdown of BRD4 in mice has been shown to have a number of effects, including depletion of stem cells from the small intestine [15]. Both platelet and gastrointestinal toxicities are believed to be on-target and are reversible upon removal of the BET inhibitor or restoration of BRD4 expression. Clinically, variation of dosing regiments has been used with some preliminary success [16] to obtain an improved therapeutic window.

In addition to the recent advancement of the clinical applications of small-molecule BET inhibitors, the field is continuously emerging with new approaches outside of the concept of small-molecule reversible inhibition of BRD4. These new strategies can potentially lead to a better understanding of BET inhibition, including clinically relevant safety parameters as well as access to more efficacious drug candidates.

BRD4 protein degradation — towards novel therapeutics

Recently, new reports describing the use of proteolytic targeting chimera (PROTAC)-induced degradation of BRD4 protein as a potential therapeutic strategy have been published. Since BET bromodomain proteins have a relatively long half-life, they are well-suited for targeting by degradation. The PROTAC methodology has been utilized previously to study protein function and target validation [17,18]. In this case, a heterofunctional molecule containing a pan-selective small-molecule BET inhibitor and an ubiquitin ligase recognition module are connected via a flexible linker. The small-molecule BET inhibitor can effectively bind to BET proteins, disrupting their interactions with acetyl-lysine of histones and transcriptional factors. This leads to the displacement of BET proteins from chromatin and is followed by ubiquitination and robust degradation of BET proteins by the proteasome (Fig. 1a). Protein degradation or PROTAC has been described previously as a successful alternative strategy to inactivate several therapeutic targets like androgen receptor (AR) and estrogen receptor (ER) through their complete degradation [17,19].

The Crews lab [20] have successfully designed a proteolytic targeting/BET inhibitor chimera, ARV-825, based on the previously developed small-molecule BET inhibitor OTX-015. Structurally, OTX-015, which is closely related to JQ1, another thienotriazolodiazepine, contains a sidechain that can be modified without significant loss of activity. This allows for the introduction of the ligase recognizing element via a suitable covalent linker. ARV-825 was designed by introducing the E3 ubiquitin ligase celebron recognizing moiety connected through an ethylene glycol linker to the solvent-exposed phenol group of OTX-015, with minimal loss of affinity for BET proteins. ARV-825 demonstrated a rapid and prolonged BRD4 degradation in several Burkitt's lymphoma cell lines, which was further translated into significant prolonged downregulation of the MYC oncogene and its downstream target genes. In comparison, treatment with small-molecule BET inhibitors, JQ1 and OTX-015, led to the accumulation of BRD4 and only partial MYC inhibition, which was not sustained upon compound removal. Importantly, ARV-825 had a superior durable effect on cell proliferation and induced apoptosis in comparison to the small-molecule reversible BET inhibitors JQ1 and OTX-015. ARV-825 did not distinguish between BRD2/3/4 and effectively degraded all protein from the BET family.

Recently, the Coleman lab [21] have demonstrated the potential utility of PROTAC-induced BET degradation against prostate cancer. It has been shown previously that BET proteins directly interact with the AR, and this interaction can be disrupted by BET inhibitors, which translates into the suppression of AR signaling, thus building a strong therapeutic rationale for patients with metastatic prostate cancer [22–25]. Applying the PROTAC strategy, the Coleman lab used the heterofunctional molecule ARV-771 (an optimized version of ARV-825), containing a JQ-1 analog (a triazolo-diazepine similar to OTX-015) as the BET inhibitor element. The BET inhibitor was linked to a von Hippel-Lindau (VHL) E3 ligase recognizing moiety (Fig. 1). Optimized VHL ligands have recently been described to have improved drug-like properties for future therapeutic applications [18,26]. With ARV-771, the Coleman lab have demonstrated efficient degradation of BET proteins in several prostate cancer cell lines at concentrations less than 1 nM, resulting in further depletion of downstream MYC mRNA and protein levels. ARV-771 was further shown to inhibit proliferation and induce apoptosis in prostate cancer cell lines 10–500 times more potently than JQ1 or OTX-015. Additionally, ARV-771 also has been shown to inhibit AR variants (FL-AR and AR-V7), leading to the downregulation of AR signaling. Importantly, *in vivo* depletion of BET proteins induced by ARV-771, administered daily for up to 20 days, led to a remarkably superior efficacy in several CRPC mouse xenograft models, causing tumor regression in the 22RV1 prostate cancer model without significant body weight loss.

A similar strategy was published by the Bradner lab [27], who utilized a hybrid degrader of BET proteins, dBET1, by coupling the small-molecule BET inhibitor JQ1 via a linker to

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