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RESEARCH HIGHLIGHT

BETs abet Tam-R in ER-positive breast cancer

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Epigenetic modifications such as histone acetylation play a central role in the transcriptional regulation of many oncogenic drivers. Accumulating evidence suggests that pharmacological modulation of certain key epigenetic reader proteins such as BRD2/3/4 may serve as an attractive strategy for treatment of many cancers, including tamoxifen-resistant breast cancer.

Estrogen receptor (ER)-positive breast cancer represents approximately 70% of all breast cancers and the selective ER modulator (SERM), tamoxifen, remains the mainstay of treatment in pre-menopausal women [1]. Although several studies have established the role of tamoxifen in significantly reducing disease recurrence and death from breast cancer, acquired resistance to tamoxifen remains a major clinical challenge [1]. Several signaling cascades have been implicated in the development of endocrine therapy resistance, including HER2, MAP kinase, PI3K-mTOR, IGF-IR and FGFR pathways [2]. However, the role of epigenetic alterations of ER in tamoxifen resistance is poorly understood.

The bromodomain and extraterminal domain (BET) family of proteins is comprised of BRD2, BRD3, BRD4 and BRDT and play a critical role in transcriptional regulation by binding to acetylated lysines on histones and recruiting general transcription factors and epigenetic regulators [3, 4]. The discovery of small-molecule inhibitors of BETs, such as JQ1 and I-BET762, has spurred intense interest in dissecting the role of these proteins in various pathological processes, including cancer [3, 4]. Recently, O'Malley and coworkers reported that the BET protein BRD3/4 plays a key role in tamoxifen resistance by recruiting WHSC1 (also referred to as MMSET or NSD2), a histone H3K36 methytransferase, to the *ESR1* gene and positively regulating its expression [5]. The authors carried out a small-scale siRNA screen against several histone methyltransferases and demethylases and identified WHSC1 as a key epigenetic enzyme that was critical for maintaining estrogen signaling in ER-positive cells. Interestingly, WHSC1 formed a positive feedback regulatory loop with ERα and was overexpressed in breast cancer. The authors established that the BET protein BRD3/4 physically interacts with WHSC1 through its N-terminal region containing two bromodomains (BD). They proposed that BRD3/4 recognizes acetylated lysines on histone tails of the *ESR1* promoter and recruits WHSC1, which promotes *ESR1* transcription by H3K36 methylation. Consistent with this model, knockdown of BRD3 and BRD4 severely compromised the recruitment of WHSC1 to the *ESR1* promoter. Interestingly, tamoxifen-resistant (Tam-R) derivatives of several ER-positive cell lines were found to be more sensitive to JQ1 treatment than their parental cells and JQ1 treatment abrogated the recruitment of BRD3/4 and WHSC1 to the *ESR1* promoter of Tam-R MCF7 cells. JQ1 treatment also resulted in persistent suppression of *ERα* mRNA levels in Tam-R MCF7 cells while recovery after prolonged treatment with JQ1 was observed in the parental cells. While the molecular basis for the sustained suppression of ERα signaling and increased sensitivity of Tam-R cells to JQ1 (over parental cells) is unclear, the authors suggested that differences in the expression of other transcription factors such as MYC and GATA3 may contribute to these effects. Nevertheless, in Tam-R mouse xenograft studies, JQ1 moderately inhibited tumor growth as a single agent but showed remarkable and synergistic antitumor activity when combined with fulvestrant, a selective ER degrader (SERD). The findings from this pre-clinical study provide impetus for clinical evaluation of BET inhibitors in tamoxifen-resistant breast cancer.

The effectiveness of BET inhibitors in tamoxifen-resistant breast cancer parallels our recent report of their effectiveness in castration-resistant prostate cancer (CRPC) [6], which analogous to tamoxifen-resistant ER-positive breast cancer, continues to maintain steroid hormone dependence. We have shown that the N-terminal region of BRD2/3/4 containing the BD1-BD2 domains physically interacts with the N-terminus of the Androgen Receptor (AR) and that this interaction is disrupted by JQ1. Furthermore, JQ1 nearly completely abrogated the recruitment of BRD4 to genomic loci shared with AR. In addition, BET inhibition also negatively regulated the expression of TMPRSS2-ETS gene fusion products and MYC. Using *in vivo* xenograft models of CRPC, we have demonstrated that JQ1 was significantly more effective than MDV3100 (Enzalutamide), a second-generation AR antagonist used clinically to treat advanced CRPC, in inhibiting tumor growth. Since the most common resistance mechanisms of endocrine therapy in prostate cancer arise due to aberrations of AR [7], the BET inhibitor-mediated abrogation of AR signaling downstream of the recep-

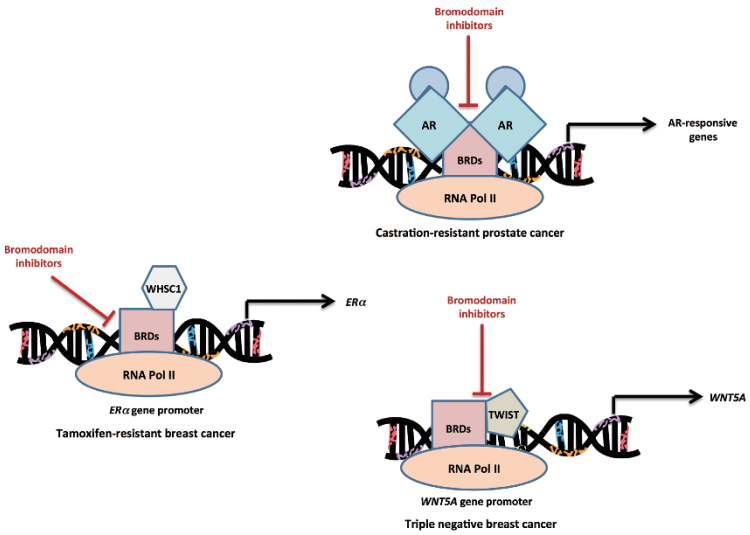

Figure 1 Context-dependent roles of BRD proteins in breast and prostate cancers. Bromodomain proteins play a key role in transcriptional regulation by interacting with acetylated histones and oncogenic drivers such as WHSC1, AR and TWIST. BET inhibitors cause preferential loss of BRD proteins at "super-enhancers" associated with key oncogenic drivers and may have therapeutic benefit in the treatment of tamoxifen-resistant breast cancer, triple negative breast cancer and castration-resistant prostate cancer.

tor has profound clinical implications in developing a durable treatment for CRPC. Since several BET inhibitors are currently in various stages of clinical development, we anticipate that our findings will spur prospective clinical trials to evaluate the efficacy of BET inhibitors in CRPC.

The most obvious intersection in the signaling pathways between breast and prostate cancers is represented by the luminal androgen receptor (LAR) subtype of triple negative breast cancer (TNBC), which is characterized by the expression of AR but the absence of ER and PR expression and Her2 amplification [8]. The LAR subtype is sensitive to anti-androgen therapy and a phase 2, open label clinical trial evaluating the safety and efficacy of MDV3100 as a single agent in patients with advanced AR-positive TNBC is currently underway (NCT01889238). Based on the findings of our study in CRPC, we predict that BET inhibition will serve as an attractive strategy for treatment of LAR subtype of TNBC, although this remains to be validated in a prospective clinical trial.

Interestingly, BET inhibition has also been reported to be effective in TNBCs that do not overexpress AR. Shi *et al*. [9] have shown that Twist, a transcriptional activator involved in inducing epithelial-mesenchymal transition (EMT), contains a "histone H4-mimic" motif that binds to BRD4 following diacetylation by Tip60, an histone acetyltransferase. The diacetyl Twist-BRD4 interaction has been shown to be necessary for constitution of an active WNT5A promoter and mediation of tumorigenicity and invasion in basal-like breast cancer (BLBC). Pharmacologic inhibition of Twist-BRD4 interaction by BET inhibitors, thus, impedes tumor growth by antagonizing the oncogenic function of Twist.

In summary, BETs, while initially characterized in relatively rare cancers such as NUT midline carcinoma [10] and acute leukemias [4], play divergent, but context-specific roles in the progression of many cancers. It is quite remarkable that although BETs are expressed ubiquitously, we found little or no toxicity in mice treated with up to 50 mg/kg daily dose of JQ1 for 30 days. While the molecular basis for such preferential toxicity to tumors remains to be understood, it has been shown that BET inhibition results in preferential loss of BET proteins at super-enhancers associated with key oncogenic drivers [11]. Although the identity of the oncogenic driver varies depending on the context, the addiction of tumors to BET-mediated activation of oncogenic pathways appears to be pervasive in a number of cancers, including tamoxifen-resistant breast cancer and CRPC (Figure 1). With the race to develop clinical grade BET inhibitors heating up, interest in the role of BETs in various cancers shows no signs of abatement.

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