Editorial

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Epigenomics

Targeting the BET family for the treatment of leukemia

> “…bromodomains of the BET subfamily promote aberrant gene expression in leukemia and their inhibition by small molecules is a promising therapeutic approach for fighting the disease”

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Sometimes serendipity is rewarded with the finding of new therapeutic targets, as has been recently shown with the discovery of BET bromodomains. Cells of various cancer types take advantage of epigenetic mechanisms to enable unchecked cellular growth. New therapeutics which reset these uncontrolled signaling pathways give hope for fighting against some untreatable diseases.

Gene expression can be regulated by chromatin modifications and DNA methylation in the underlying epigenetic code, the epigenome. Cancer cells are often characterized by altered epigenetic landscapes, which are known to exploit the chromatin regulatory machinery to induce aberrant gene expression and abnormal cell proliferation [1]. In this context, histone modifications have a huge potential in the therapeutic treatment of epigenetically driven diseases. Histone modification proteins include ‘writers’, ‘erasers’ and ‘readers’ [2]. The last comprise, among others, the well-studied bromodomain (BRD) family, which specifically recognizes ε-*N*-acetylated lysine residues. The human genome encodes up to 61 different BRDs present in transcriptional co-regulators and chromatin-modifying enzymes, including the bromodomain and extraterminal domain (BET) subfamily. It is composed of eight different domains present in four homologous proteins, namely BRD2, BRD3, BRD4, and BRDT, which modulate gene expression by recruiting transcriptional regulators to specific genomic locations [3]. BRD2 and BRD4 have crucial roles in cell cycle control of mammalian cells. Along with BRD3, they are functionally linked to pathways important for cellular viability and cancer signaling and are also co-regulators in obesity and inflammation [4]. Thus, small molecules that inhibit BET BRDs have a very promising potential as antiinflammatory and anticancer agents. Members of the subfamily have also been postulated as targets for latent HIV-1 reactivation and for the development of male contraceptives [5,6]. Anticancer activity is mainly due to down-regulation of the key oncogene MYC [7]. Cytotoxicity in lung adenocarcinoma cell lines by BRD4 inhibition has been additionally linked to suppression of the oncogenic transcription factor FOSL1 and its targets [8].

Currently, two 1,4-diazepine derivatives, namely (+)-JQ1 and I-BET, are in preclinical development in cancer and inflammation, respectively, as potent antagonists of the BET bromodomains BRD2, BRD3 and BRD4 [9,10]. Furthermore, the BET BRD inhibitor RVX-208 increases transcription of the apolipoprotein A1 gene and is in Phase II clinical trials for atherosclerosis and diabetes, and OTX015 is in Phase I trials for acute myeloid leukemia (AML) and other hematologic malignancies [11]. Fragment-based screenings and QSAR-based lead optimizations for the discovery of small molecules with BRD4 inhibitory activity have shed a few new relevant chemical scaffolds featuring sulfonamide moieties, including compound 6a, an isoxazole derivative at an initial developmental stage in clinics, and the 3-methyl-3,4-dihydroquinazoline-2-one PFi-1 [2,12,13]. We have recently presented a pioneering

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large-scale structure-based virtual screening campaign in the epigenetics field. It comprised more than 7 million compounds, which were rationally reduced to a set of 24 molecules and subsequently experimentally tested to validate their *in vitro* affinity for BRD4. The experiment yielded seven novel compounds with significant inhibitory activity against the target, including XD14, a 4-acyl pyrrole derivative that specifically and potently inhibits BET BRDs [14]. X-ray crystallography indicated that the molecule disrupts the interaction of BRD4 with acetylated lysines by occupying its recognition site as well as a new region of the binding pocket addressed for the first time by a small molecule (PDB ID: 4LYW).

> "...the next generation of BET bromodomain inhibitors could actually arise from an apparently unrelated field: the kinase inhibitors."

There is strong evidence supporting the hypothesis that BRDs of the BET subfamily promote aberrant gene expression in leukemia and that their inhibition by small molecules is a promising therapeutic approach for fighting the disease. Indeed, the BET BRD inhibitor XD14 showed specific, potent antiproliferation activity against leukemia cells in a panel containing 56 diverse cell lines representing nine different cancer classes [14]. Ott *et al.* could show through *in vitro* and *in vivo* experiments that BET bromodomain inhibition results both in a potent suppression of MYC transcription and activity, and a dramatic decrease of expression of the cytokine receptor IL7R in B-cell acute lymphoblastic leukemia (ALL) [15]. In the case of mixed lineage leukemia (MLL), the BET BRD inhibitor I-BET151 has been used to perturb the interaction of the polymerase-associated factor complex (PAFc) and the super elongation complex (SEC) disease-relevant BET complexes with chromatin, eventually proving its efficacy in *in vivo* murine models and primary patient samples of MLL-fusion leukemia [16]. BRD4 has also been identified as a therapeutic target in AML, being critically required for disease maintenance [17]. Researchers could show that the BET BRD inhibitor JQ1 leads to antileukemic effects *in vitro* and *in vivo*, along with terminal myeloid differentiation and elimination of leukemia stem cells [17]. Similar sensitivities were observed in a variety of human AML cell lines and primary patient samples, highlighting the robustness of the approach. In an independent experiment, a treatment of only 2 days with the BET BRD inhibitor JQ1 on the human erythroleukemia (HEL) cell line UT7 was sufficient to transitory inhibit Epo-induced UT7 cell proliferation and to restore terminal erythroid differentiation [18].

The enormous therapeutic potential of BET BRDs has benefited an unprecedented interest in the discovery of novel inhibitory strategies. Yet, the next generation of BET BRD inhibitors could actually arise from an apparently unrelated field: the kinase inhibitors. Strikingly, it could be shown that the cyclin-dependent kinase inhibitor dinaciclib, which recently advanced to Phase III clinical trials for the treatment of leukemia, interacts with the acetyl-lysine recognition site of the testis-specific BRDT [19]. Such a finding opens a new era in the design of BET BRD inhibitors by mining the vast chemical space of kinase inhibitors, even stimulating potent, selective second-generation dual BRD-kinase inhibitors. Furthermore, this strategy could be bidirectional: the highly specific BET BRD inhibitor I-BET151 has shown efficacy against Janus kinase 2 (JAK2) dependent myeloproliferative neoplasms, inducing significantly overlapping transcriptional changes with a JAK2 inhibitor in HEL cells [20]. Investigation on the potential duality of other BET BRD inhibitors, e.g., XD14, is tempting.

Herein, we have presented compelling recent scientific evidence supporting BRDs of the BET family as therapeutic targets that play an important role in the development and maintenance of several types of commonly diagnosed leukemia, including AML, ALL, and MLL. As BRDs recognize and attach to altered histones, the disruption of this interaction by BET BRD inhibitors has proven effective in *in vitro* and *in vivo* leukemia models. Currently, only few potent and selective BET BRD inhibitors have been described, but some of them have already found their way into clinical trials for the treatment of therapeutic diseases. Second-generation dual BRD-kinase inhibitors could enhance the already very bright future of known BRD modulators.

Researchers are just beginning to understand the complex and dynamic processes involved in the chromatin regulatory machinery, and only a minute portion of the epigenetic proteins have been studied to a drug-discovery meaningful extent. Current efforts have focused mainly on histone acetyltransferases, histone deacetylases (HDACs), BRDs, as well as DNA methyltransferases (DNMTs). Pioneering experimentation has already provided inspiring success stories in the epigenetics field: apart from the two chemotherapeutic DNMT1 inhibitors azacitidine and decitabine, the histone deacetylase inhibitors vorinostat and romidepsin have been licensed by the US FDA for the treatment of cutaneous T-cell lymphoma [11,21]. Provided with their very promising therapeutic potential, BET BRD inhibitors could become marketed drugs as well during the next few years. It is worth mentioning that the well-studied BET BRD subfamily represents

only eight domains out of the 61 BRDs encoded in the human genome. Therefore, a broader, thorough analysis of the yet unknown biological role and therapeutic potential of other BRDs will reveal further crucial targets for fighting against untreatable diseases.

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