New Direction

Research has shown that BET inhibition has an impact on many different biologies and could demonstrate potential as a novel, epigenetic approach to treating a variety of illnesses such as cancer, inflammation and, most recently, cardiovascular disease. Success in this area may mark a shift towards a new paradigm for drug development

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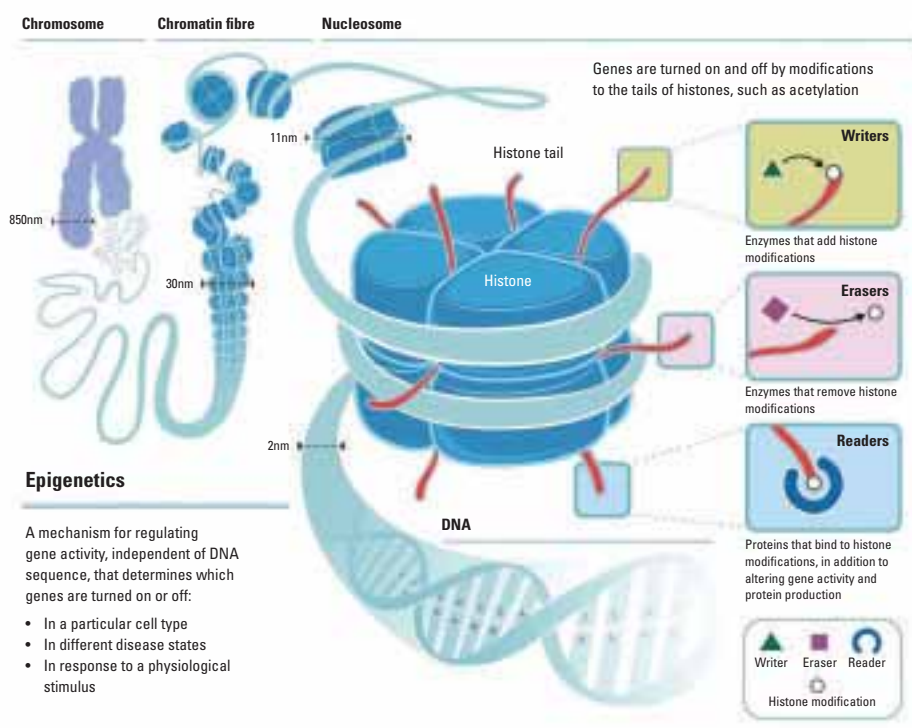
Epigenetics describes the mechanisms by which chromatin-associated proteins and post-translational modifications regulate gene expression. These heritable traits, not linked to changes in the DNA sequence, refer to the epigenetic code or pattern of post-translational modifications on proteins and DNA that lead to the regulation of transcription (1). The role of the epigenetic regulators and transcription factors in maintaining the genome in open or closed conformation, thereby controlling the transcriptional programme, is fundamental to cellular processes such as proliferation, development and differentiation. Dysregulation of epigenetic mechanisms is closely linked to the progression of disease.

Figure 1: Epigenetics: a mechanism for regulating gene activity, independent of DNA sequence



Readers, Writers and Erasers

Epigenetic control over transcriptional programmes facilitates appropriate and specific cellular function within each tissue and organ system, in spite of a common genomic sequence shared by every non-germline cell in the body. Adding (writing) and removing (erasing) post-translational modifications – for example, acetylation, methylation and ubiquitination – to the protein components of chromatin is followed by 'reading', which dictates gene expression and eventual phenotypic response (see Figure 1) (2,3). Through this mechanism, cellular programmes are tightly regulated, yet responsive to stimuli.

Inappropriate epigenetic activity can lead to cellular dysfunction, and is implicated in a variety of diseases, such as cancer and autoimmune disorders; epigenetics is therefore a promising avenue for research. The first of these therapies to reach the clinic have been histone deacetylase and DNA methyltransferase inhibitors (4). They have been approved for the treatment of cutaneous T cell lymphoma and myelodysplastic syndromes, and continue to be investigated in numerous drug development programmes targeting writers and erasers.

The development pipeline is also starting to fill with drugs targeting readers, or the proteins that recognise the pattern of post-translational modifications and guide gene expression. Readers – such as those within the bromodomain class of proteins – recognise and bind acetylated lysine residues on histone tails, found in actively transcribing

regions of chromatin (5), and serve as a docking platform for the assembly of large transcriptional complexes and the recruitment of key transcriptional proteins, like positive transcription elongation factor (p-TEFb) (6). This bromodomain-containing family, consisting of approximately 46 diverse nuclear and cytoplasmic proteins, has been the focus of significant drug development efforts in the oncology, autoimmune and vascular disease arena (7).

BET Proteins

Bromo and extraterminal (BET) proteins make up a sub-group of the 46 bromodomain-containing proteins and consist of four members: BRD2, BRD3, BRD4 and BRDT. All four BET proteins contain two conserved N-terminal bromodomains (BD1 and BD2), which recognise and bind acetylated lysine residues on histone tails and other nuclear proteins (8). These interactions localise BET proteins to discrete locations along the chromosome, where they recruit and facilitate assembly of factors to influence gene expression (9). BET proteins regulate genes that play a part in proliferation, cell cycle progression and apoptosis (9,10).

Dysfunction of these proteins has been associated with the development of aggressive tumours. Notably, nuclear protein in testis (NUT) midline carcinoma (NMC) arises as a result of a fusion protein between the N-terminal bromodomain of BRD3 or BRD4 with NUT. In the first pivotal studies in this space, a BET inhibitor, JQ1, was shown to bind to and target the bromodomain aspect of this fusion protein responsible for driving the oncogenic transformation in the tumour (11). By way of its inhibition, the fusion protein was released from chromatin, shutting down the oncogenic gene expression and validating BET proteins' roles in transcriptional programmes.

By recognising chromatin conformation (post-translational modifications), BET proteins can contribute to pathological gene expression. This role is even more magnified in states of aberrant lysine acetylation, where chromatin is in a constitutively open, transcriptionally available conformation. Reading by BET and other bromodomain-containing proteins can, therefore, contribute to this inappropriate gene expression, as has been demonstrated with genes such as c-Myc (12) and Aurora B (13). Importantly, based on findings showing the presence of BET proteins at super-enhancer sites driving the expression of oncogenes, regulating the function of these readers is an attractive strategy for shutting down expression in a variety of cancers (14). Consequently, BET inhibition is a valid therapeutic strategy in oncology, as well as other states of dysregulated gene expression (15).

Clinical Studies in Cancer

Findings from preclinical studies have fuelled interest in the field of epigenetic drug development, with sights set on new and improved small molecules for BET inhibition. Based on the protein-protein binding between BET proteins and acetylated lysines present in the chromatin, small-molecule inhibitors are being avidly pursued. Notably, several different, orally available, low molecular weight scaffolds and molecules have been developed – a number of which have moved into the clinic and are in early clinical trials, including:

- I-BET762/GSK525762A – Phase 1 in relapsed, refractory haematologic malignancies, as well as NUT midline carcinoma and other cancers (clinicaltrials.gov identifiers: NCT01943851 and NCT01587703)
- TEN 010 – Phase 1 in acute myelogenous leukaemia, myelodysplastic syndromes and advanced solid tumours (clinicaltrials.gov identifiers: NCT02308761 and NCT01987362)
- OTX015 – Phase 1 in acute leukaemia, other haematological malignancies and selected advanced solid tumours (clinicaltrials.gov identifiers: NCT01713582 and NCT02259114); and Phase 1/2 in recurrent glioblastoma multiforme and newly-diagnosed, acute myelogenous leukaemia (clinicaltrials.gov identifiers: NCT02296476 and NCT02303782)
- CPI 0610 – Phase 1 in progressive lymphoma, multiple myeloma, acute myelogenous leukaemia, myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms (clinicaltrials.gov identifiers: NCT01949883, NCT02157636 and NCT02158858)

Effects on Multiple Biologies

Based on their function in binding acetylated residues on histones and other proteins, and facilitating assembly of transcriptional complexes, bromodomain-containing proteins play a role in regulating transcription. This regulation extends beyond oncogenes and modulates a variety of cellular programmes inherent to correct cell functioning and environmental response. For instance, regulation of the transcriptional programme upstream of pro-inflammatory mediators is one of the prime mechanisms governing inflammatory diseases and states. NF-κB is a key regulator of the immune response, and its part in directing transcription of downstream mediators involves the recruitment of NF-κB co-activators, such as BRD4 (15,16). It is therefore not surprising that BET inhibitors have been shown to potently suppress the inflammatory transcriptional response.

A recent study by Brown *et al* has extended these observations by investigating the genome-wide relationship between these two factors in endothelial cells (17). The findings demonstrated that a subset of NF-κB functions occur through super-enhancer complexes, which can be influenced by BET inhibition. In fact, JQ1 administration significantly attenuated disease progression in a mouse model of aberrant inflammation (in atherosclerosis), thus inferring the important role of BET proteins, and the potential for BET inhibition in diseases with an inflammatory component, such as atherosclerosis, sepsis or autoimmune diseases (15,18).

Beyond effects on NF-κB, BET inhibition has demonstrated a role in the differentiation and activation of TH17 cells – as well as the pro-inflammatory functions of TH1 cells – and likely has a role in other immune responses (18,19). BET proteins have also been identified as central co-activators of transcription factors driving aberrant gene expression in heart failure and pathological remodelling of the heart. Treatment with BET inhibitors arrested hypertrophy and heart failure in murine models, providing a rationale for developing these inhibitors as therapeutic agents in heart disease (20). BET family members have additionally been implicated in the regulation of viral genome replication and expression of viral proteins. This holds therapeutic potential in the activation of latent viruses such as HIV (21). In doing so, the awakened virus can be targeted with antiviral agents, making BET bromodomain inhibition a viable strategy for addressing and eradicating HIV.

Upstream of many transcriptional programmes, BET inhibition may be a new frontier for regulating and modulating multiple cellular biologies simultaneously, which could provide therapeutic benefit for many diseases.

BETs in Other Indications

The most advanced BET inhibitor in clinical development is RVX-208. This compound was originally discovered in a screen for Apolipoprotein A1 (APOA1) mRNA inducers in hepatocyte cell cultures, and has been shown to raise APOA1 and high-density lipoprotein (HDL) in humans (22). RVX-208 binds preferentially to the second bromodomain of BET family members, with a 20-fold or higher selectivity for the second bromodomains of BRD2, BRD3 and BRD4 versus the first bromodomain (23,24). This inhibition modulates expression of a variety of genes including APOA1 – the core protein component of HDL (15,23,24). Elevating APOA1 increases reverse cholesterol transport, which is essential for atherosclerotic plaque regression in the treatment of high-risk cardiovascular disease patients. RVX-208 has been shown to raise the body's *de novo* synthesis of APOA1 and HDL by this mechanism in a number of Phase 1 and 2 clinical trials (25), in indications related to cardiovascular disease (CVD) and metabolic disease (clinicaltrials.gov identifiers: NCT01728467, NCT01067820, NCT01423188, NCT01058018 and NCT00768274).

In post hoc analysis of the SUSTAIN and ASSURE trials, pooled data analysis demonstrated statistically significant increases in APOA1 and other markers of reverse cholesterol transport (26). Beneficial effects were also observed on CVD biomarkers, such as high-sensitivity C-reactive protein, alkaline phosphatase, and components of the complement and coagulation cascades. RVX-208 treatment also had an effect on the incidence of major adverse cardiovascular events (MACE); defined as composite of death, non-fatal myocardial infarction, coronary revascularisation procedures, and hospitalisation for unstable angina or heart failure. Data from this analysis demonstrated a 55% relative risk reduction (RRR) in the incidence of MACE in CVD patients, and a more pronounced 77% RRR in patients with a history of diabetes mellitus. Taken together, the combined effects of BET inhibition on multiple biologies known to impact vascular risk may, in part, explain the reduction in MACE events – a promising finding for this class of compounds.

Based on its mechanism of action, BET inhibitors such as RVX-208 may have additional utility in indications related to its effects on multiple biologies – for example, chronic kidney disease, Alzheimer's disease, heart failure, peripheral artery disease and other immune-related disorders.

New Paradigm

Epigenetics is a novel and promising area of research with the potential to simultaneously modulate multiple biologies. Molecular and mechanistic studies have already provided exciting insight into the function of BET inhibitors, and indicate a possible shift from the 'one target-one drug' development model, to a more physiologically relevant approach of concurrently modulating multiple biological processes, all of which contribute to the diseased state. BET inhibitors may therefore be at the forefront of this new paradigm for drug development.

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