

EPIGENETICS

are we hitting the mark?

During the last few years, advances in the understanding of epigenetic processes have led to an explosion in interest in this area as a potential source of new targets for the discovery of medicines. In addition, development of chemical probes directed toward specific players in histone modification is showing promise as a way to help unravel the highly complex ways in which epigenetic systems impact gene expression. Via this work, the potential for whole new classes of therapeutic targets are emerging, driven in large part by the ability to discover and optimise compounds which modulate epigenetic systems in specific ways. In this commentary, we highlight the ways in which compound screening approaches are being successfully applied to epigenetic targets and place these into perspective relative to more traditional drug targets.

Within the field of epigenetics, considerable attention is now moving from basic science towards exploring potential applications in medicine. One exciting aspect of this work is the possibility for emergence of whole new classes of drug target which are highly distinct from those which have been the traditional focus of drug discovery (eg G-protein coupled receptors, Ion channel, kinases, proteases, nuclear receptors etc). The pursuit of entirely new types of drug target poses a number of interesting questions and challenges. For example, how do screening methods and chemical libraries which were developed for traditional drug targets perform when applied to epigenetics? Are there fundamental differences in the ways in which these targets are being approached as a result of the scientific and technological advances available now compared to earlier eras of drug discovery? In most cases, it seems too early to answer these questions, but enough progress has been made that it is worthwhile highlighting some emerging themes in terms of drug discovery approach, challenges and successes.

In this article we will not describe in detail what is known about epigenetic processes, nor provide a detailed overview of targets or compounds. Many excellent reviews have been published which cover this material [see Bibliography/background reading].

Epigenetic modulator compounds

There are currently two classes of drugs approved for clinical use which target epigenetic mechanisms, DNA methyl transferase (DNMT) inhibitors (eg Vidaza, decitabine) and histone deacetylase (HDAC) inhibitors (eg Vorinostat). Both classes of agents are used to treat specific cancer types and act via fairly non-specific mechanisms (ie broadly inhibit these classes of enzymes as opposed to targeting specific enzyme sub-types). Both were discovered and developed in an era in which understanding of epigenetic processes was in its infancy.

There is still interest in finding compounds which modulate DNA methylation via a more targeted approach than current DNMT inhibitors (for example by targeting specific DNMT isoforms). However, the majority of attention is now

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Table 1: Summary of the major classes of Epigenetic target

TARGET CLASS	FUNCTIONAL ROLE	TARGET SUB-TYPES	APPROX CLASS SIZE
DNA Methyl transferase (DNMT)	DNA methylation to silence gene expression	Multiple isoforms	4
Histone Methyl transferase (HMT)	Installation of methyl marks on histone H3/H4	Lysine and Arginine transferases	60
Histone De-methylase (HDM)	Removal of methyl marks from histone H3/H4	JmjC and Lysine-specific demethylases	32
Methyl mark reader domains	Sensing of methyl marks on histone H3/H4	PHD, Chromodomain, Tudor, MBT, PWWP, WD40	199
Histone Acetyl transferase (HAT)	Installation of acetyl marks on histone H2-H4	GNATs, P300/CBP, MYST, TF-related, NR coactivators	18
Histone Deacetylase (HDAC)	Removal of acetyl marks from Histone H2-H4	Classes I-IV	18
Acetyl Mark Reader domains	Sensing of acetyl marks on histone H2-H4	Bromodomain, tandem	61

focused on the mechanisms by which histone tails within chromatin are modified at specific residues. This includes a wide range of proteins that regulate (and respond to) histone methylation, acetylation, ubiquitination and phosphorylation and thus represent a rich potential source of new drug targets (Table 1). These modulators of the histone code¹ comprise many enzymes and binding proteins that are involved in ‘writing’ (ie catalysing the addition of a specific histone mark), ‘erasing’ (removing a mark) or ‘reading’ (detecting the presence of a specific mark to trigger an effect on gene expression) post-translational modifications (PTMs) of histone tails.

The potential complexity of the histone is staggering (there are around 10³⁰ possible permutations of histone PTMs). Through these mechanisms cells are able to become exquisitely specialised via highly controlled expression of certain sets of genes. These systems are also dynamic and affected by cellular environment. Clearly, modulating the balance of histone marking within cells via functional manipulation of key epigenetic players is likely to have profound effects and therefore is of strong interest from a drug discovery perspective. Drug safety will also need to be approached cautiously with these approaches, given the widespread potential for modulation of gene expres-

sion. Cancer cells are fundamentally dysregulated with respect to gene expression and so, unsurprisingly, much of the current work in this area is focused on oncology. However, modulation of gene expression in specific cell types has the potential to be useful in many therapeutic areas.

One particularly intriguing aspect of this emerging area is the potential to drug whole new classes of biological target that have been previously unexploited. A good analogy is with research on protein kinases which have been the subject of discovery activities now for some 25 years or so. *In vivo* kinases need to act in very specific ways via recognition of substrate and spatio-temporal control of their activity (incidentally, epigenetic processes have exactly the same requirement). However, the basic catalytic mechanism of transferring a phosphate group from ATP to a protein amino acid residue is conserved. This has a number of important consequences which help increase the efficiency and success rate of discovering new compounds that potently and specifically inhibit kinase activity. First, as kinases all catalyse the same type of reaction, albeit with different substrates, assay technologies and methods can be rapidly applied from one enzyme to the next via a platform approach. Second, conservation of the protein active site topology means that chemical connectivity can be

exploited to discover inhibitors for additional kinases based around a set of design principles (or collection of pharmacophores) created from previous experience. This again provides a platform approach which, in effect, lowers the risk and cost of exploiting each additional target within the class. This type of approach has driven protein kinase research to the point where CROs can offer routine services in which compounds can be profiled against, essentially, the entire kinome, allowing for good predictions of *in vivo* specificity.

The major families of proteins which create and remove histone marks are fairly large (Table 1) and, like kinases, do have conserved mechanisms so could potentially be exploitable in similar ways. Hence, considerable effort is now being focused both in pharma and academic groups on the creation of chemical and assay platforms as a means to launch the discovery of bio-active compounds which target epigenetic processes and provide ways to understand the biological consequences of such modulation.

Currently, there is a significant challenge in selecting specific epigenetic proteins as drug targets due to a lack of target validation and pre-existing evidence of chemical tractability. Target validation is achieved with evidence that modulation of the function or expression will have a disease modifying effect in a safe and effective way. Chemical tractability, on the other hand, is evidence that supports the prospect for discovery and optimisation of compounds which will perform the desired function in a safe and effective way. Despite advances in genomics and development of gene silencing methods such as siRNA, these two factors are often closely linked. That is, the most credible means of target validation requires the use of a tool compound in a cellular or *in vivo* experiment, where generation of such a tool compound answers the question of chemical tractability! This type of consideration has at least two related consequences. Firstly, the initiation of new drug discovery efforts against novel biological targets tends to be very risky if the tractability of the target is unknown and validation is dependent upon the successful outcome of the tractability assessment. Secondly, more tractable (especially if this is part of a protein-class platform) targets typically require less biological validation prior to initiation of inhibitor discovery efforts, since there is an expectation that useful compounds can be discovered in a cost and time-effective manner at the outset.

It is for reasons such as these that screening methods are at the centre of the emergence of what might be referred to as 'applied epigenetics'. The

current attempts to open up new classes of target for exploitation by chemical biology and/or drug discovery comes at a particularly interesting time, given the relative maturity of high throughput screening methods and instrumentation and the evolution of academic screening.

Lead discovery approaches as applied to epigenetic targets

So, what methods are being predominantly used to discover epigenetic modulators and do these differ from those used for other more traditional target classes? While there are a number of epi-modulator compounds currently under clinical development, these are relatively limited and a much better sense of what approaches are being successfully used can be obtained from looking at earlier stage molecules, often referred to as chemical probes.

Given the rapid emergence of the field of epigenetics and in particular a recent focus on histone methyl-transferases and demethylases as potential targets, there is now a range of good assay methodologies available². Following selection and optimisation of an appropriate assay, what distinguishes screening approaches is the selection of compound libraries to be tested. Based on this selection, screening can be broadly broken down into four categories, all of which are being actively applied to epigenetic targets:

- 1. Diversity screening (aka HTS).** In this approach, targets are tested against a compound collection optimised to have desirable chemical properties and which attempts to cover as much chemical diversity as possible. As such, the selection of compounds is biased towards properties which are generally attractive, not towards a specific target or set of targets. The sizes of diversity screening sets vary considerably (from around 50,000 to several million compounds). Recently, new methods which utilise DNA-barcoding (Encoded Library Technologies; ELT)³, have allowed huge scaling of diversity library sizes.
- 2. Focused screening.** In this approach, the selection of compounds is biased towards activity against a specific set of targets. In principle, it should be enriched in active compounds and thus provides a more efficient way of obtaining starting points.
- 3. Knowledge-based drug discovery.** Here, specific knowledge of the target or (more likely) compound interactions with the target is used to design or select a set of compounds directed toward the specific target.
- 4. Fragments.** This method uses small chemical fragments, generally around 200Da, to identify

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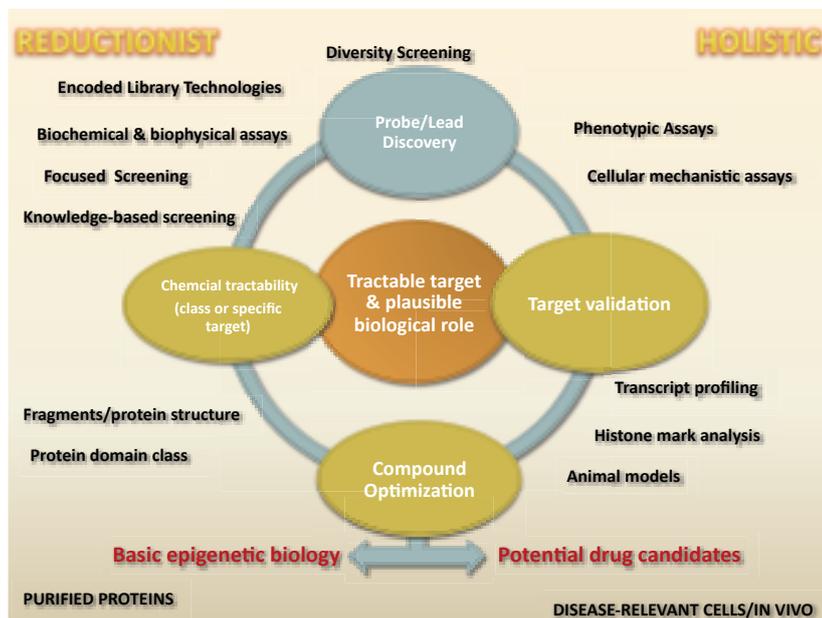


Figure 1
Schematic representation of some key steps and approaches in the pursuit of epigenetic targets

weak binders of target proteins. Compounds are optimised by growing fragments to increase affinity. The smaller size of these fragments allows testing of greater chemical diversity with smaller numbers of compounds when compared to HTS.

All of these methods have been applied now to a relatively wide range of targets representing all of the major classes of epigenetic targets. In some cases (eg histone methyl-transferases) there are preliminary indications that focused library sets might provide an efficient means to obtaining useful chemical starting points⁴. Similarly, there are now quite a few reports of success using knowledge-based approaches, largely driven from protein structural information. As far as it is possible to tell from the quite limited literature so far, diversity screening approaches appear to be the most common way in which groups are approaching screening these targets, and there have been a number of reports of success. There is also some anecdotal evidence from all of these approaches that targets appear to vary widely in their chemical tractability. Clearly, development of approaches which allow early and relatively inexpensive assessment of tractability would be valuable in these cases. On the flip side, the screening approaches and, to a large extent, chemical libraries and methods developed for use against more traditional drug targets, do seem to work well for some epigenetic targets. Hence the long-term prospects for further exploitation via chemical biology methods and/or conventional drug discovery are positive.

Phenotypic and cellular mechanistic hit ID

The linkage of particular epigenetic marks to histone modifying enzymes provides opportunities for cellular mechanistic screening. Monitoring changes in several PTMs in cells has been achieved using fluorescently labelled antibodies, ALphaLISA technology and TR-FRET detection coupled with transient expression of GFP-histone substrates. However, enzymatic activity sufficient for disease-associated biological consequences may be restricted to histones associated with specific promoter regions and thus not observable as a global change of a particular mark. In addition, lack of quality antibodies, slow catalysis, low histone turnover and enzymes with redundant activities may further hinder development of cellular assays that measure changes in specific epigenetic marks.

Cell-based reporter assays have been developed to overcome these challenges, mainly in the field of HDAC inhibitor discovery. Reversal of aberrant silencing of tumour suppressors can be measured in assays that couple reactivation of an epigenetically silenced promoter region to transcription of a reporter gene. One advantage to this approach is that recruitment of transcriptional repressors, in addition to enzymatic activity, is retained as a mode of inhibition.

The use of pure phenotypic assays has been limited in epigenetic drug discovery despite the proapoptotic and anti-proliferative activity associated with inhibition of many epigenetic targets. The exceptions include the discovery of the HDAC inhibitor Vorinostat as an inducer of murine erythroleukemia cell differentiation and the benzodiazepine inhibitors of BET family bromodomains identified as anti-inflammatory compounds in a cellular apolipoprotein A1 (ApoA1) reporter HTS⁵. The relatively poor understanding of epigenetic pathways and dependence on cellular environment has so far hindered the widespread use of phenotypic assays as a hit identification strategy for histone-modifying enzymes and proteins.

Genome-based phenotypic screening may provide opportunities for identifying novel small molecule regulators of epigenetic pathways. Instead of a single-minded focus on changes in a particular epigenetic mark, compound effects on transcript profiles for specific downstream cellular outcomes may provide better starting points for targeting histone-modifying enzymes in their true biological context. High-throughput transcription-based profiling is well suited for epigenetic targets as their mechanism generally involves changes in transcription due to changes

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in histone tail modifications of associated chromatin. This approach may also reveal previously unidentified modes of action for marketed drugs that exploit epigenetic mechanisms.

Reductionist and chemical probe approaches

While the drive to screen targets within a context that is as close to the *in vivo* (and disease) conditions via phenotypic assays is attractive, it does not readily enable systematic exploration of specific sets of proteins or protein domains with respect to chemical tractability. The use of chemical probes is an important strategy for addressing the problems of target identification and defining the biological role of players in epigenetic processes.

This type of approach is exemplified by the work conducted within the Structural Genomics Consortium (SGC) and collaborating institutions⁶. The method is built upon relatively high throughput (and common across protein class) platforms to express and assay specific types of target, to allow highly efficient and parallel prosecution. A similar platform approach may be utilised with screening methods such as ELT. This methodology allows very large DNA-encoded compound libraries to be interrogated using small amounts (10s of ug) of protein and is amenable to large scale, parallel screening of panels of proteins⁷.

There is a strong emphasis by the SGC on solving protein crystal structures, including co-crystals with probes bound, that facilitates knowledge-based design approaches. Such approaches could also potentially be directed not only towards the active/binding site of proteins, but also protein:protein interactions which direct the spatio/temporal specificity of the interactions of epigenetic players with chromatin⁸. Protein structural information is also a key driver for fragment-based screening approaches (see above). Edfeldt and colleagues recently postulated that the success of screening fragments may be a strong indicator of the chemical tractability of targets⁹. It therefore seems logical that this type of approach may be used more extensively in the future to unravel which epigenetic targets are druggable, sometimes in advance of existing knowledge linking specific targets to disease.

The chemical probe approach also benefits strongly from the development of focused libraries directed towards specific target types. Along with substrate-mimetic compounds such as SAM, published and newly discovered inhibitors of epigenetic targets are being assembled to form libraries used to explore potential new targets.

Whatever the method used, the output of these approaches would typically be compounds which are relatively potent (<1uM), selective and have appropriate properties to be active in cellular models – for example showing a significant change in a specific histone mark. While there is no guarantee that the compounds discovered using this type of approach will result in the initiation of drug discovery efforts, they hold value even if the outcome is to exclude a target from further interest (for example, if the compounds demonstrate target-specific toxicity). At the very least, chemical probes help clarify the role of their target protein by facilitating pharmacological modulation of activity. In addition to demonstrating and exemplifying chemical tractability, they also bootstrap subsequent hit identification efforts.

Future prospects, key issues and challenges

The field of applied epigenetics must still be regarded as in its infancy, but promising progress has been made against many of the classes of potential epigenetic target. Thus far, it seems that the screening methods and chemical libraries used for more traditional drug target classes can be successfully applied to epigenetic targets, albeit with variable success. The reasons why, even within a specific class of targets (eg histone methyl transferases), certain targets seem quite tractable whereas others appear recalcitrant to all screening methods is not understood at present. This might have to do with the basic architecture of the proteins, how they are being screened, or even some fundamental factors about how they operate *in vivo*.

A considerable amount of effort both within industry and academia is being directed towards applied epigenetics and this is yielding a considerable toolbox of bioactive compounds. This will allow the promise of perturbing epigenetics processes as a therapeutic approach to be explored deeply in the coming years. While the promise of epigenetics is considerable, it is also likely to present some interesting challenges. One obvious question is how to ensure the safety of epigenetically-directed drug candidates given the complexity and centrality of epigenetic control of gene expression. Drug intervention at the level of epigenetic targets may also be predicted to result in a phenomenon which could perhaps be called 'sub-acute pharmacology', since pharmacologic (and toxicologic) action is not dependent simply upon exposure to the drug, but rather the temporal nature of protein expression under epigenetic control. Hence the pharmacodynamics of drug effects may well be

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determined as much by the recovery of the ‘tone’ of gene expression upon wash out of the drug.

A wide range of screening methods, ranging from holistic phenotypic approaches through to broad panning for chemical probes and evidence of tractability across protein class, are being applied successfully to the field of epigenetics. The outputs from these efforts seem likely to play a key role both in the discovery of medicines and the understanding of basic biological processes. **DDW**

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