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Epigenetic opportunities and challenges in cancer

Jonathan D. Best and Nessa Carey

CellCentric, Chesterford Research Park, Little Chesterford CB10 1XL, UK

Epigenetic covalent modifications of DNA and chromatin proteins strongly affect gene expression and cellular activity, and epigenetic misregulation occurs in several diseases, especially cancer. First-generation drugs targeting the relatively promiscuous DNA methylation and histone acetylation modifiers have had successes in the treatment of haematological cancers. Second-generation drug programmes are in the discovery phase, targeting epigenetic enzymes with more tightly defined modes of action. This review highlights some of the challenges in identifying the most appropriate new targets and the issues that need to be addressed to facilitate the successful entry of second-generation epigenetic drugs into the clinic.

The term 'epigenetics' is one that has been interpreted in several ways, differing in subtle and not-so-subtle details. A useful working definition is the one provided by a recent conference review: 'an epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence' [1]. Two aspects of epigenetics, as exemplified by this definition, have particular attraction in the field of cancer therapeutics. The first is that the trait is heritable and, hence, would be maintained in rapidly dividing cancer cells. The second is that the trait is mediated at the chromatin level but not via genetic micro- or macro-mutations. A consequence of this is that the heritable phenotype, which in cancer would be miscontrolled cell growth and/or proliferation, could be inhibited by modulating the cellular machinery responsible for the trait and 'liberating' the underlying genetic information (e.g. re-expression of an aberrantly repressed tumour suppressor protein).

Functionally, epigenetics refer to a network of covalent modifications to DNA and histone proteins, which, in turn, interact with other cellular proteins to regulate gene expression. A top-level schematic of this process is shown in Fig. 1. Modification of DNA is almost exclusively a consequence of methylation of cytosine residues, whereas histone modifications show a much greater diversity. Several outstanding reviews have described the range of histone modifications that have been identified [2–6]; a brief overview is presented in Table 1, and additional diagrammatic information in given in Fig. 2. As implied by the diagram, the majority of histone modifications occur in the relatively accessible N-terminal tail region, rather than in the histone body. A full review of the modifications is outside the scope of this review, but several features should be highlighted. DNA methylation is considered the most robust of chromatin modifications and is associated with gene silencing, and most researchers are now comfortable with the theory that DNA methylation can act as a causal factor in transcriptional inactivation [7–11]. The widely explored modification of histone acetylation is considered a 'fuzzy' modification – broadly speaking, chromatin regions in which histones are heavily acetylated are transcriptionally active, whereas the converse is true for deacetylated sections of the genome [12]. Histone methylation and the other modifications listed in Table 1 are believed to be far more finely tuned, with more subtle but just as far-reaching impacts on gene expression. For example, the histone mark H3K4me3 (trimethylation of histone H3 at the lysine 4 residue), positioned in the promoter regions of genes, is generally associated with transcriptionally active chromatin, whereas the H3K9me3 modification is usually a mark of inactive chromatin [13,14]. The diversity of marks has given rise to the concept of a histone or epigenetic code [15–20]. However, we remain a long way from being able to read this code in a genuinely predictive way. This is partly because the technologies required to perform the necessary global cellular analyses at high epigenome coverage have only been developed recently (see e.g. Ref. [21]) and also because of the high degree of complexity and cross-talk between the modifications, some of which are transient and all

E-mail address: nessa.carey@cellcentric.com.

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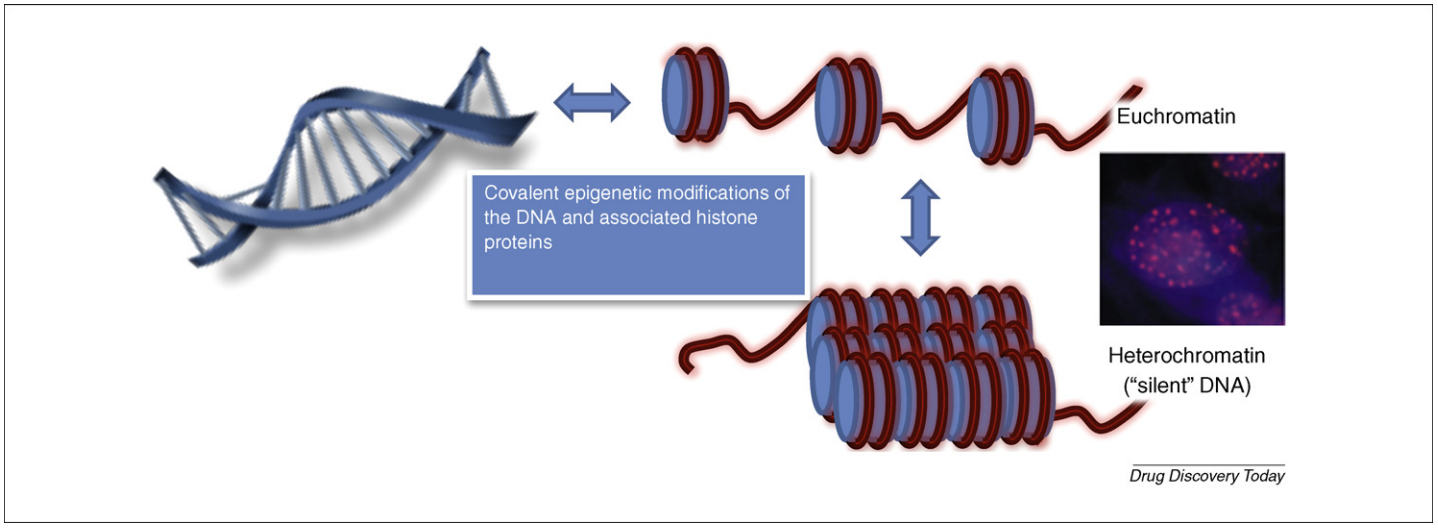


FIGURE 1

A schematic of epigenetic processes. These are essential for packaging and interpreting the genome and are responsible for the establishment of global chromatin environments and the orchestration of DNA-based functions (gene transcription, DNA replication, DNA repair and chromosome condensation).

of which impact on the likelihood of other modifications becoming established and influencing gene expression and cell fate.

Despite the fact that publication rates in epigenetics show exponential year-on-year growth, numerous questions remain unanswered. These include the mechanisms underlying active DNA demethylation and how histone marks are transmitted from one cellular generation to the next.

Epigenetics and cancer

There is an extraordinary degree of complexity in chromatin modifications and signalling, yet there is substantial enthusiasm for the development and implementation of epigenetic therapies in several human disease areas, especially oncology. What has led to these high expectations? Broadly, it is a combination of three factors: axiom, experience and prediction.

TABLE 1

The major classes of epigenetic enzymes

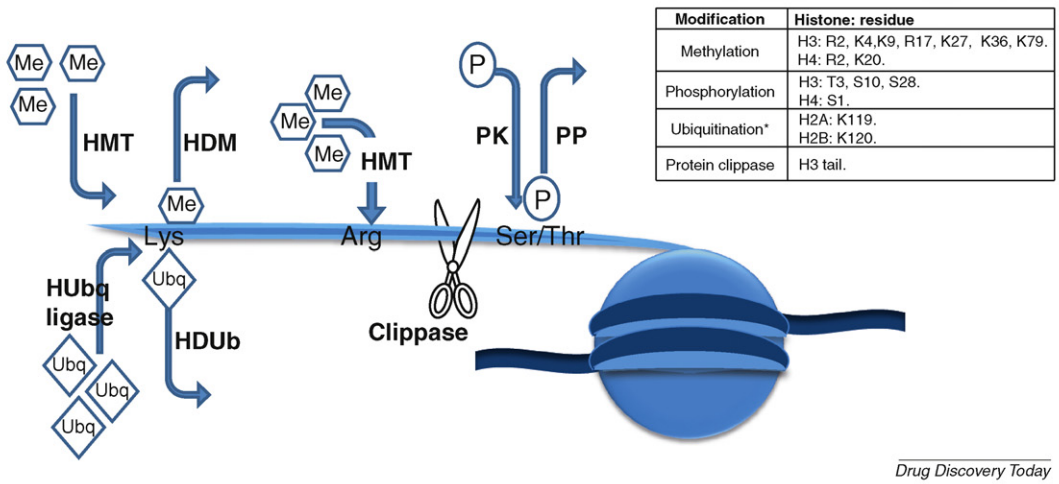
Enzyme class	Function
Acetyltransferase	Adds acetyl groups to lysine residues
Deacetylase	Removes acetyl groups from lysine residues
Methyltransferase	Adds methyl groups to lysine or arginine residues
Demethylase	Removes methyl groups from lysine or (possibly) arginine residues
Ubiquitin ligase	Adds ubiquitin group to lysine residues
Deubiquitinase	Removes ubiquitin group from lysine residues
Protein clippase	Removes a small number of tail amino acids
Ribosyltransferase	Adds one or more ADP and ribose moieties
Convertase (deiminase)	Converts arginine to citrulline
Kinase	Adds phosphate groups to serine or threonine residues
Phosphatase	Removes phosphate groups
Sumo ligase	Adds small ubiquitin-like modifiers to lysine residues
Sumo protease	Removes small ubiquitin-like modifiers from lysine residues
Proline isomerase	Converts *cis*-proline to *trans*-proline

Axiom

The uncontrolled proliferation of cells that is one of the hallmarks of cancer requires a constant dysregulation of the cellular homeostatic mechanisms that operate in healthy cells. One of the mechanisms by which this is achieved is abnormal signalling from growth factors (reviewed in Ref. [22]), and therapies aimed at this signalling have proven utility (recent reviews include Refs. [23–27]). Yet in most of these cancer types, remission and/or relapse is a frequent occurrence because eventually, a population of cancer cells develops in which cell growth and division have become independent of such signalling. In essence, this implies a re-setting of the gene regulatory mechanisms, such that abnormal expression has become the default state and is maintained at a favourable 'cost' to the cell. The most obvious ways in which this can be achieved are by genetic and/or epigenetic stabilization of gene expression and, hence, cellular function. Reversal of genetic change remains too challenging a hurdle (e.g. there is little scope with current technologies for correcting an inactivating somatic mutation of a tumour suppressor gene). By contrast, the disruption of epigenetic misregulation via enzyme inhibition has considerable pharmacological attraction.

Experience

There are two major classes of epigenetic therapies in the clinic for oncology indications – DNA methyltransferase (DNMT) inhibitors and histone deacetylase (HDAC) inhibitors, as shown in Fig. 3. Although it was believed initially that cancer was associated with DNA hypermethylation and, hence, gene inactivation, it is important to recognize that this hypermethylation occurs against a background of general DNA hypomethylation (for a recent review, see Ref. [28]), and it is probable that the targeted nature of the increased DNA methylation levels is a consequence of an initially more selective histone-mediated transient silencing of key tumour suppressors [29].



Modification	Histone: residue
Methylation	H3: R2, K4,K9, R17, K27, K36, K79. H4: R2, K20.
Phosphorylation	H3: T3, S10, S28. H4: S1.
Ubiquitination*	H2A: K119. H2B: K120.
Protein clippase	H3 tail.

FIGURE 2

Actions of histone-modifying enzymes. HDM, histone demethylases; HMT, histone methyltransferases; HUbq ligase, histone ubiquitin ligases; HDUB, histone deubiquitinases; PK, protein kinase; PP, protein phosphatase; Me, methyl groups; P, phosphate groups. Inserted table shows the residues known to be modified on the various histones. *Ubiquitination refers to the signalling processes mediated through mono-ubiquitination rather than polyubiquitin-mediated proteasomal degradation.

The anti-proliferative effect of HDAC inhibition was a serendipitous discovery, and to some extent, this group of ubiquitously expressed enzymes remains a surprising drug target. The compound trichostatin A (TSA) was shown to have an anti-proliferative effect long before it was shown to be an HDAC inhibitor [30]. Although TSA itself is not a suitable compound for treating humans, it provided the insights required to drive the development of safer and more efficacious HDAC inhibitors. This is reflected in the large number of commercial HDAC inhibitor programmes that have been initiated (Fig. 3).

Prediction

The DNMT inhibitors and HDAC inhibitors have blazed a trail for epigenetic therapies but are far from perfect. Their clinical successes have mainly been against haematological cancer, and they show little consistent efficacy against solid tumours [31–37]. The reasons for this are unclear. It might be important that as a class, HDAC enzymes are promiscuous, in that they target all acetylated lysine residues in histone tails. The majority of the more recently discovered histone epigenetic modifiers are far more specific in their actions. For example, LSD1 demethylates H3K4me1 and

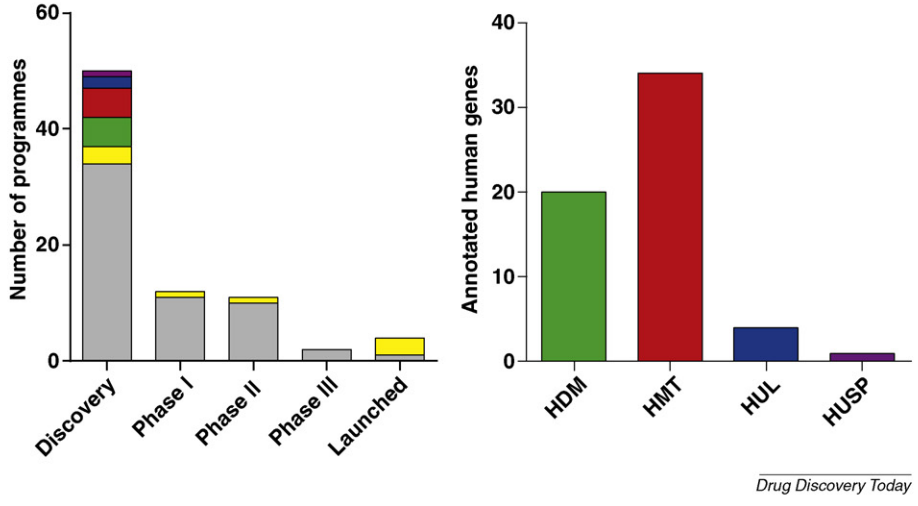


FIGURE 3

Profiles of epigenetic gene classes and epigenetic drug discovery and development programs. **(a)** Development profile of epigenetic programmes. Grey, HDAC inhibitors; yellow, DNA methyltransferases; green, histone demethylases; red, histone methyltransferases; blue, histone ubiquitin ligases; violet, histone ubiquitin-specific proteases. *Source*: Thomson Pharma, epigenetic company websites (Data derived from open sources including NCBI Entrez and PatentLens). **(b)** Number of annotated human genes returned from NCBI human gene search. HDM, histone demethylases; HMT, histone methyltransferases; HUL, histone ubiquitin ligases E3; HUSP, histone-ubiquitin-specific proteases (search terms used column headings and *Homo sapiens*; the search was restricted using the limits to include current records and RefSeqs and manually curated).

H3K4me2 but not H3K4me3, or indeed methylated H3K9 [38]. The general consensus amongst epigenetic scientists is that greater successes are likely to come from targeting histone-modifying enzymes with less promiscuous activities than the HDACs. Importantly, several epigenetic enzymes have been reported to be overexpressed in certain cancers [39–43], and certain histone modifiers have been shown clearly to act as tumour suppressors or cellular oncogenes (e.g. Refs. [44,45]).

Second-generation cancer epitherapeutics – the challenges

As shown in Fig. 3, DNMT and HDAC inhibitors are already licensed for certain indications, but programmes targeting the enzymes that perform the more subtle chromatin modifications are all in the discovery phase. This is an inevitable consequence of the pace at which epigenetic targets have been identified: DNMTs were being extracted from mammalian livers by the early 1970s [46], yet LSD1, the first enzyme shown to demethylate methylated histones, was only identified in 2004 [38]. The rapid progress that is taking place in epigenetics is one of the features that not only makes it of such interest in drug discovery and development, but also makes it exceptionally challenging. The challenges for the pharmaceutical industry lie in all three of the classically related areas of biology, chemistry and drug development.

Biology

The most fundamental question in creating new therapeutic opportunities for cancer treatment is target selection, and epigenetics is no exception to this. Even just concentrating on four classes of enzymes that have been shown to modify histone proteins (the methyltransferases and demethylases and the ubiquitin ligases and deubiquitinases), it is obvious from Fig. 3 that there are multiple potential candidates in each class. Indeed, the data in Fig. 3 raise an interesting question – are there more programmes on histone demethylases than histone deubiquitinases because the former are an intrinsically 'better' class of targets, or does this simply reflect current (and probably imperfect) knowledge? Is the pharmaceutical industry focussing on particular enzyme classes not because those are the most fundamentally important in cancer but simply because these are the ones about which most is known? If the latter is true, have the current epigenetic targets really been rationally selected or have they been almost self-selected?

Why is target selection in epigenetics so difficult? The problem is not only in the large number of potential targets but also in their complex interactions. The epigenome is, by definition, a more dynamic landscape than the genome, and this creates difficulties in identifying the most appropriate intervention points in cellular function. A recent publication [47] demonstrating a complex interaction of cycles of ubiquitination and deubiquitination of human histones provides one such example, wherein the addition and subsequent removal of mono-ubiquitin molecules signals to other epigenetic modifiers and mediators of gene regulation.

It is this dynamic set of relationships that accounts for the ongoing drive to develop inhibitors of, for example, both histone methylation and demethylation. Although at first glance this could seem counterintuitive, it reflects our deepening understanding of the interactions between different histone marks. Consider the example of a tumour suppressor gene that has been inappropriately silenced in a tumour by epigenetic mechanisms. The H3K9me3 mark is associated with transcriptional repression. In this case, inhibition of the methyltransferase that generates this modification would be predicted to have a positive effect on expression of the tumour suppressor. Conversely, the H3K4me3 mark is an activating mark, and inhibition of the demethylase that removes this modification would also act to stabilize the active conformation.

It is also probable that certain epigenetic events will be more important in some cancer types than in others, and it is far from clear whether our current selection methods are sufficiently comprehensive to capture all opportunities. Certain enzymes might be excluded as candidates if there is clear evidence they act as tumour suppressors (e.g. the histone demethylase FBXL10 [48]) or if naturally occurring inactivating mutations are associated with cancer initiation and/or progression, as has been recently reported for UTX, another demethylase [49]. For others, knockdown experiments might indicate that an enzyme has a proliferative role, as has been shown for LSD1 [50] or the histone methyltransferase G9a [51]. Unfortunately, the interpretation of knockdown experiments might be complicated by the presence of multiple functional domains in a protein because they will not show which domain is the crucial one for mediating an anti-proliferative readout. For example, the histone demethylase JARID1C contains a DNA-binding ARID (A/T-rich interaction domain) domain and two PHD zinc finger-like motifs, in addition to the two Jumonji domains that mediate the hydrolysis reaction underlying demethylation [52]. Multiple functional domains are common in epigenetic enzymes where interactions with chromatin and other chromatin-modifying/binding proteins are an integral part of their function.

In summary, identification of the most promising candidates will require inputs from a variety of experimental disciplines and integration of data from several laboratories to optimize truly rational target selection.

Chemistry

Epigenetic targets present several challenges for the development of small-molecule inhibitors. Not least is that many of these targets are in enzyme classes that are poorly covered by existing therapeutic molecules. Although this might seem superficially attractive – it would suggest large areas of the chemical landscape should be available for exploitation – this can make identifying good starting points for extensive chemistry programmes to develop safe and efficacious drugs considerably more difficult. One of the reasons why so many companies have created HDAC inhibitor programmes, for example, is that they have been able to build out from known chemical starting points such as TSA and sodium butyrate. There are far fewer opportunities for this kind of approach in the second-generation epigenetic classes. Pharmacological inhibitors have been reported for the G9a histone methyltransferase [53], which might act as starting points for this enzyme class. The same is also true for LSD1, in which polyamine inhibitors have been reported in the literature [54], but in this case, it is much less clear that this specific class of molecules will really serve as appropriate starting points for successful medicinal chemistry programmes. Crystal structures exist for several potential epigenetic targets, including JMJD2A and PR-SET7/9 [55,56], and these might also aid drug discovery efforts.

There inevitably remain substantial numbers of epigenetic targets with promising biological datasets for which there are no existing

chemical inhibitors and no reported crystal structures. Identification of appropriate chemical hits might be more challenging for these enzymes and might require high-throughput screening of compound libraries.

Development

There is a well-defined crucial path for getting anti-cancer compounds into the clinic in terms of the regulatory environment and the need for demonstration of efficacy and safety. What is much less clear is how to implement this path for new epigenetic therapies, a problem that is intimately related to the relative immaturity of the field. Several areas are highlighted below but cannot be comprehensive in a review of this length.

There is currently no consensus on how important the issue of compound selectivity will be in the application of epigenetic therapies. The majority of HDAC inhibitors are pan-inhibitors, and although at least one company has dedicated considerable resources in an attempt to develop selective HDAC inhibitors, it is not clear that these would have clinical advantages for either the safety or the efficacy aspect [57]. The situation is just as unclear for the newer epigenetic targets. If targeting histone demethylases, for example, should a drug development programme aim for absolute selectivity (e.g. JARID1C over other JARID subfamily members), for selectivity for one class of histone demethylases over another (e.g. JARID family members over more distantly related Jumonji proteins) or for general selectivity (e.g. for histone demethylases over related enzymes, such as prolyl hydroxylases)? It might prove difficult to determine the importance of selectivity until late in the discovery cycle when data become available on the efficacy and toxicity of candidate drug compounds.

Which are the appropriate patient groups for the application of epigenetic therapies? At the moment, this remains unclear and is an issue that must be resolved if second-generation epigenetic targets are to reach their full potential. Although it would be possible to follow the example of HDAC (and other non-epigenetic programmes) and predominantly attempt to address this issue in clinical trials, there are several disadvantages to this approach. It is ultimately very expensive and has no guarantee of success – the second-generation epigenetic drugs might be considered failures if they do not show efficacy in solid tumours. Currently, our understanding of the fundamental biology of these exciting new targets is inadequate for making these assessments, but it is improving. There are data suggesting that some histone-targeting enzymes also modify p53, for example [58,59], and this might provide a mechanistic route for patient stratification. The relative precision of these targets, compared with DNMT and HDAC enzymes, might also prove advantageous because it might be possible to identify cancer types and/or patient populations in which the epigenome itself provides data on the most susceptible groups.

The success of second-generation epigenetic therapies might also be influenced by subpopulations of cells within a tumour. Some of the most promising epigenetic targets were originally identified not because of a role in cancer but because they are of major cellular importance during very early developmental stages in the mammalian embryo that are characterized by the presence of pluripotent and/or multipotent cells. This raises the tantalizing theoretical possibility that the same epigenetic enzymes might be involved in a similar way in the maintenance of a relatively undifferentiated cancer cell with characteristics of a stem cell. The existence of such cancer stem cells remains controversial, at least in solid tumours [60]. If cancer stem cells do exist, they might present not only an additional opportunity for therapies based around epigenetic modifiers but also an additional challenge because the drug development pathway for compounds that would be aimed at a residual stem cell population, rather than the majority of rapidly cycling cells, is far from clear.

An essential prelude to patient selection will be the identification of appropriate pharmacodynamic markers, to underpin preclinical proof-of-action and proof-of-mechanism studies. These might be surprisingly complex; in live cells and intact tissue, many epigenetic modifications will be in a state of flux. For example, analyses of deubiquitinases in cells often have to be conducted in the presence of pharmacological ubiquitin ligase inhibitors, or the latter will mask the effects of the former.

A logical extension of this is that epigenetic inhibitors might work not just as single agents but as components of combination therapies. This should not be taken just to mean the existing strategy in oncology development in which new compounds are added to existing therapeutic regimes of, for example, cytotoxic drugs but in the targeted development of combination therapies underpinned by strong hypothesis-driven research. An example would be the simultaneous use of two compounds, one an inhibitor of a specific histone methyltransferase and the other an inhibitor of a specific histone demethylase, as described above. This would require not just a change in preclinical testing of compounds but, ultimately, pressure on regulatory authorities to change the way in which clinical trials are designed and implemented to gain the greatest benefit from scientific breakthroughs.

Concluding remarks

Epigenetic control of gene regulation is a rapidly developing field of substantial potential, and oncology is likely to be the therapeutic application in which the fastest progress is made. However, if knowledge, know-how and clinical expertise remain in isolated silos, as is currently the case, epigenetic therapies will continue to reach the market but in an *ad hoc* and inefficient manner, which will fail to meet fully the needs of industry or patients. A more coordinated approach, involving the integration of disparate research methodologies and findings from the entire field, is required to realize the therapeutic potential of epigenetics more fully.

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