Epigenetic therapies for non-oncology indications

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Chronic and degenerative disorders are a major, and growing, human health burden, and current treatments are in many cases inadequate or very expensive. Epigenetic therapies are attractive options for treating such disorders because they manipulate the processes that maintain cells in an abnormal transcriptional state. The challenges lie in identifying the most appropriate diseases and the enzymes that should be targeted. This review describes the different approaches that can be used to address this problem, focusing particularly on CNS disorders (especially mental retardation, neurodegenerative disease, psychiatric disorders and drug addiction), diabetes and diabetic complications, and autoimmunity and inflammatory diseases.

Epigenetic traits have been defined operationally as ‘stably heritable phenotypes resulting from changes in a chromosome without alterations in the DNA sequence’ [1]. Epigenetic modifications (also known as epigenetic marks) form a network of covalent alterations to DNA and histone proteins, which, in turn, interacts with other cellular proteins, typically in multi-component mediator complexes. The end result is the regulation of gene expression. This regulation can be short-term and dynamic or exceptionally stable if the chromatin modifications lead to the hypermethylated DNA state associated with the formation of transcriptionally silent heterochromatin. Several excellent reviews cover the underlying modification mechanisms [2–4], and Fig. 1 summarizes our current knowledge of these modifications. It is impossible in one figure to demonstrate the complexity of histone modifications present on even one histone molecule at a single genomic locus in a single cell. In some cases, modifications are mutually exclusive (e.g. it is not possible for a single histone H3K4 residue to be methylated and acetylated simultaneously). A single residue can be modified to varying degrees – many lysine residues can be mono-, di- or trimethylated. Different combinations of modifications can only occur in certain situations. The combination of methylation on H3K4 and H3K27 only occurs in pluripotent cells and, even then, only at the promoters of certain key regulatory genes [5].

There has been considerable progress in the development of epigenetic drugs for the treatment of human cancers [6]. DNA methyltransferase inhibitors and histone deacetylase (HDAC) inhibitors have been licensed by the US Food and Drug Administration. Several companies are now developing inhibitors for second-generation epigenetic targets, focusing on enzymes that mediate restricted histone modifications. Although oncology is the current major focus for most of these programmes, there is optimism that epigenetic drugs will have wider therapeutic applications.

It might at first seem counterintuitive that processes involved in the uncontrolled proliferation and transformation that are characteristics of cancer could be useful intervention points in non-proliferative disorders, but in reality this is not so surprising. Epigenetic mechanisms control cell fate. Aberrant epigenetic processes can have several potential outcomes, depending on the enzymes and pathways involved, the specific cell type, interactions with the environment and so on. Given the large numbers of enzymes involved in epigenetic processes, a role only in cancer would be far more surprising than an involvement in multiple indications.

New therapeutic indications

Perhaps the greatest problem in developing epigenetic drugs for non-oncology indications lies in identifying the most relevant diseases to target. In general, it might be helpful to think of
epigenetic processes serving to stabilize a response to an external stimulus, such that in disease states they act to maintain a cell in an abnormal transcriptional programme. This leads to the hypothesis that epigenetic interventions might be most useful in chronic and developmental disorders, and the limited data available support this theory. Epigenetic effects are also attractive mechanisms for accounting for discordance between monozygotic twins.

Within the broad category of chronic and developmental disorders, there are two complementary and intersecting approaches that have been useful in identifying human diseases that might be amenable to epigenetic therapies: biology (both human and animal models) and drug repositioning. Figure 2 lists some of the major disorders beyond oncology for which an epigenetic component or therapeutic approach has been proposed. Several Mendelian disorders have been shown to be the result of mutations in genes encoding epigenetic enzymes or mediators. This has been particularly fruitful in the field of mental retardation. Angelman’s syndrome and Prader-Willi syndrome were recognized many years ago as associated with parent-of-origin and imprinting deficits, extreme examples of epigenetic regulation and abnormality. Rett’s syndrome, the X-linked neurodevelopmental disorder, is predominantly caused by mutations in MeCP2, a protein that binds methylated DNA residues[7]. This protein might also be implicated in human autism[8]. Elegant work from Adrian Bird’s lab has shown the reversal of the Rett phenotype in engineered mouse strains[9]. Although this cannot yet be directly replicated in human patients, because the mouse work required a genetic approach, it suggests that neurodevelopmental defects can be reversed, offering considerable encouragement to the field.

PHF8 is a histone demethylase, and mutations in this gene have
been identified in several families with a history of X-linked mental retardation [10,11].

The majority of chronic human disease is not associated with Mendelian inheritance patterns, however, so how can the pharmaceutical industry use biology to identify other disorders that might be amenable to epigenetic therapies? One tantalizing approach is to identify epigenetic fingerprints characteristic of disease (i.e. to identify marks present in disease states that are absent in health). This is a huge undertaking, fraught with difficulties. As described above, the large numbers of epigenetic enzymes can result in an enormous number of possible combinations of modifications, and identifying those that are associated with a disorder and demonstrate a causal relationship with disease aetiology will require a step-change in detection technologies and bioinformatics. Researchers are taking steps towards this on a global scale (the Thousand Epigenomes project is one example; see http://nihroadmap.nih.gov/epigenomics), and intriguing data already exist in the literature for several disorders.

Alzheimer’s disease (AD) is marked by a loss of cholinergic neurons, along with the formation of Abeta protein plaques and neurofibrillary tangles [12]. Research into therapeutics for AD has focussed on either cleavage of the amyloid precursor protein or hyperphosphorylation of the tau protein, which forms neurofibrillary tangles [13]. Developing effective therapeutics using these approaches has met with limited success, and studies have begun to investigate epigenetic changes in animal models of AD. Regional variations in methylation and acetylation of histone proteins have been seen in the brains of the familial AD mutant Tg2576 mouse, suggesting that compounds targeting methylation and acetylation might be useful [14]. Other findings have suggested that HDAC inhibitors might play a part in ameliorating learning and memory deficits [15]. These studies are at a very early stage, however, and further work is required. This includes analyses of human samples to determine whether the same patterns of epigenetic modifications are present as in the mouse model and whether they genuinely distinguish Alzheimer’s pathology from general ageing effects. It is also necessary to investigate whether these fingerprints are causal effectors of the disease or merely markers of disease progression.

In addition to neurodegenerative disorders, epigenetics is an increasing focus in the investigation of psychiatric disorders. One of the most intensively explored has been the long-term effects of childhood abuse or neglect, which is associated with several adult health deficits including increased risk of depression, drug addiction and suicide. The most commonly used rodent model of early life stress is built around periodic mother–infant separation. The offspring maintain elevated levels of glucocorticoid secretion throughout life, and this overactivation of the hypothalamic–pituitary–adrenal axis is also found in affected humans [16]. This seems to be underpinned by epigenetic misregulation at several levels. The arginine vasopressin (AVP) protein is a key stimulator of adrenocorticotrophin release from the pituitary. A specific AVP enhancer is hypomethylated in the paraventricular nucleus of mice subjected to early life stress, and this leads to persistent overexpression of the gene [17]. Other studies have demonstrated increased DNA methylation, and concomitant decreased expression, of the neuron-specific glucocorticoid receptor promoter (Nr3c1) in the same model system [18]. Because the hypothalamic–pituitary–adrenal system is usually controlled by a positive feedback loop, the combined effects of the altered (hypo- and hyper-) methylation at each ‘end’ of the axis would be continual overstimulation of this hormonal response.
There are always questions over the applicability of animal models to human psychiatric disorders. In this instance, the Nr3c1 effects reported in the rodent model are also reported in human adult suicides with a history of childhood abuse [19]. The changes were not seen in suicides with no abuse history or in unaffected controls.

It is unclear from the literature whether the early events establishing a hypomethylated state at the AVP enhancer occur in mitotic or post-mitotic neurons. If it is the latter, it will be important to identify which, if any, of the recently identified putative active DNA demethylases are present in these cells, because there is no opportunity for passive DNA demethylation in post-mitotic cells [20–22]. At least some of the hypomethylation at the AVP enhancer is reported to be driven by the dissociation of the repressive MeCP2 protein from the locus. MeCP2 can act to drive the continued recruitment of DNA methyltransferases to a silenced chromatin region, and hence loss of this binding protein will lead to long-term hypomethylation.

MeCP2 has been reported recently to be a key protein in animal models of drug addiction. Knockdown of MeCP2 in the rat striatum led to decreased cocaine intake in an animal model of unrestricted drug access [23]. Manipulation of MeCP2 levels in the nucleus accumbens of mice altered locomotor responses to amphetamines [24]. The details of the models and the mechanisms postulated for the effects of MeCP2 vary between the two studies (for discussion, see Ref. [25]), but both are supportive of a major role of DNA methylation. Additional support for the importance of DNA methylation in response to drugs of addiction comes from the finding that manipulating levels of the DNMT3a DNA methyltransferase in the nucleus accumbens of mice markedly affected their response to cocaine [26].

DNA methylation is an exceptionally stable epigenetic modification and might be difficult to manipulate for psychiatric disorders because even the licensed DNA methyltransferase inhibitors are unlikely to have side-effect profiles that would be acceptable for these indications. Many gene responses become initially transiently stabilized via histone modifications before more permanent DNA methylation changes are established. These histone modifications might, in the future, become useful targets for the development of drugs that target acute stresses, to prevent long-lasting psychiatric disturbances such as post-traumatic stress disorder.

Moving away from psychiatric disease, the HDAC inhibitors MS-275 and SAHA (vorinostat) have been shown to relieve pain in the second phase of the formalin rat pain model, causing upregulation of the brain mGlu2 receptors. This suggests a role for histone acetylation in the transcriptional activity of the mGlu2 receptor gene [27].

Neurological disorders are not the only diseases in which there is an ongoing interest in epigenetic therapies. There are expected to be 285 million cases of diabetes worldwide in 2010, 90% of which will be Type 2. This number is increasing, predominantly because of lifestyle changes (http://www.diabetesatlas.org). Affected individuals have an increased risk of cardiovascular disease and a wide range of other pathological conditions, creating a major human health burden. Currently, primary management of both Type 1 and Type 2 diabetes centres on glycaemic control and insulin therapy, along with weight control strategies and the use of statins and treatments for hypertension [28–30]. Early studies have begun to show links to epigenetic effects, both in the insulin release and insulin response pathways themselves and in the inflammatory pathways, which are mediators of morbidity and mortality. Hyperacetylation of histone H4 under high-glucose conditions has been reported at the insulin gene promoter in the insulinoma cell line MIN6. DNA hypomethylation of the same promoter was shown in the beta cells of the pancreas in murine and human samples. Experimental methylation of this promoter suppressed expression of the insulin gene [31]. Vascular endothelial cells cultured under hyperglycaemic conditions showed persistently increased levels of the activating H3K4me1 mark in the promoter of the pro-inflammatory NF-κB-p65 gene. This was probably mediated by the SET7 methyltransferase [32]. In the same study, it was observed that levels of the inhibitory H3K9me2 and H3K9me3 marks at the same promoter were reduced. Investigations of the inflammatory aspects of diabetes demonstrated the involvement of H3 methylation in the inflammation of vascular smooth muscle [33,34], where H3K9me3 was purported to play a part in the repression of inflammatory genes [34]. High glucose levels were seen to increase the expression of inflammatory genes and the levels of H3K4me2, reducing the recruitment of the repressive histone demethylase LSD1 [33]. Several additional studies have demonstrated the association of histone H3 methylation events with diabetic end-points, indicating that histone methylation and acetylation pathways could both be potential candidates for small-molecule epigenetic therapeutics in this disease [35,36].

In addition to Type 1 diabetes, several autoimmune diseases are being investigated for underlying epigenetic mechanisms, particularly systemic lupus erythematosus, rheumatoid arthritis (RA) and multiple sclerosis [37]. Changes in histone and DNA modifications are associated with inflammatory responses [27,38,39]. Decreased DNA methylation at a specific CpG motif in the IL-6 promoter of patients’ peripheral blood mononuclear cells has been linked to RA [40]. Systemic lupus erythematosus patients have been reported to have decreased DNA methylation in the promoter of the CD5-E1B gene, a key regulator of the same interleukin [41]. In both clinical conditions, the end-point of these DNA methylation events increased the expression of IL-6, a key inflammatory cytokine.

The alternative approach for identifying disorders that might be amenable to epigenetic therapies is a repositioning strategy. Drug repositioning is the process by which drugs (usually marketed drugs) are assessed in diseases for which they were not originally developed. The potential advantage is that marketed drugs have been through safety and toxicology testing in humans and thus can be fast-tracked into clinical trials in alternative indications [42]. In this instance, it involves epigenetic drugs originally developed to treat cancer – although ironically, from this review’s perspective, one of the first examples of such an approach happened the other way around. Sodium valproate was one of the earliest successful anti-convulsant drugs and has been widely used in epilepsy treatment. It is now known that valproic acid is a low-affinity HDAC inhibitor with anti-proliferative effects in several cancer models.

HDAC inhibitors are generally well tolerated, and many compounds have been made available to researchers working in oncology areas, using model systems ranging from fruit flies to...
mice. They have proved particularly informative in some Mendelian disease models. For example, Huntington’s disease (HD) is an untreatable and invariably fatal dominantly inherited disorder caused by an extension of CAG repeats in the Huntingtin (Htt) gene, with consequent loss of neurons from the striatum being one of the prominent pathologies [43]. There are several mouse models of this disease, of which R6/2 is generally accepted as a useful disease phenocopier. Repressed transcription is a common feature in HD tissues, and HDAC inhibitors generally act as transcriptional activators. R6/2 HD mice treated with the HDAC inhibitor SAHA showed improved motor function and increased longevity [44]. Later papers also demonstrated symptomatic improvements in HD models using the less potent HDAC inhibitors sodium valproate and phenylbutyrate [45,46]. Surprisingly, in all cases the symptomatic improvement that followed administration of HDAC inhibitors was not accompanied by changes in underlying cellular pathology, suggesting more investigation is needed to fully understand the mechanism of action of these drugs in the model systems.

EnVivo Pharmaceuticals entered an HDAC inhibitor (ENV-0334) licensed from MethylGene into phase I clinical trials with HD as a named indication. The most recent statements from the company, however, suggest that this compound is being positioned as a cognitive enhancer for other neurological indications, including AD and Parkinson’s disease (http://www.envivopharma.com/template/2_18_5.html). ENV-0334 inhibits the class I and class II zinc-dependent HDACs. Recently, an inhibitor of the class III NAD-dependent Sirtuin 1 HDAC, EX-527/SENO0014196 (generated by Elixir Pharmaceuticals and partnered with Siena Biotech), has entered phase I clinical trials for HD (http://www.sienabiotech.com/portfolio.jsp).

In addition to acetylation, recent studies have shown that the HTT protein interacts with the polycomb repressive complex 2, which possesses methyltransferase activity targeted at H3K27 [47]. The significance of this finding in the disease aetiology is unclear.

The importance of understanding the biology is further demonstrated by work on dopaminergic neuronal cell lines, where treatment with the HDAC inhibitor trichostatin A (TSA) resulted in decreased cell survival and increased apoptosis [48]. This suggested that HDAC inhibition might not be appropriate for the treatment of Parkinson’s disease. However, this is in contrast to observations of Wu et al. [49], who noted that administration of valproic acid or TSA to dopaminergic neurons in rat neuron–glia cultures upregulated GDNF and BDNF and had a neuroprotective effect against the neurotoxin MPTP. Other studies investigating the effects of HDAC inhibitors have also shown positive effects. Kim et al. [50] observed that the HDAC inhibitors sodium butyrate and TSA seemed to stimulate neurogenesis in the brains of rats with induced ischaemia. The effect was mediated by the BDNF tyrosine kinase signalling pathway. AD mouse models showed restoration of contextual memory with the chronic administration of different HDAC inhibitors [51]. This study was supported by Smith et al. [52], who demonstrated that administration of valproic acid to microglial cell lines led to increased phagocytic activity for Abeta deposits.

An intriguing application of epigenetic approaches has been the use of HDAC inhibitors to drive re-expression of developmentally silenced genes, to compensate for the loss of function in Mendelian disorders. Spinal muscular atrophy is caused by inactivating mutations in the SMN1 gene [53]. The human genome also contains a highly homologous gene, SMN2, which is usually epigenetically repressed in postnatal tissues. Treatment with HDAC inhibitors leads to reactivation of the SMN2 gene in model systems [54]. A parallel approach might be possible in inherited haemoglobinopathies. Some patients with inherited mutations in the β-globin gene that lead to either sickle cell disease or β-thalassemia fare better than expected clinically [55]. Frequently, this is due to the persistence of foetal haemoglobin gene (HbF) expression. The HbF gene is usually epigenetically repressed postnatally, but studies with HDAC inhibitors in model systems have shown that the gene can be de-repressed by drug treatment [56].

Concluding remarks

With epigenetic-focused therapeutics being a promising avenue for non-cancer indications, the questions of how to assess prospective therapeutics in vivo become key. The majority of animal models are centred around either genetic manipulations, such as the transgenic mice used to model neurodegenerative disorders [57,58], or pharmacologically induced behavioural phenotypes, such as the pilocarpine epilepsy model [59]. In monogenic disorders such as HD, the epigenetic changes seen in the animal models are likely to reflect those seen in humans, although even here the disconnect between behavioural improvements and cellular pathology leads to questions around behavioural testing and whether this is appropriate or linked closely enough to the underlying pathology [60]. For the more complex diseases such as AD, the current animal models mainly mimic inherited diseases [57], so being able to determine effects on the sporadic elements of the disease will require understanding from different mechanisms of the pathology. There is often surprisingly little consensus within the pharmaceutical industry on the most reliable animal models for the majority of complex diseases (including AD, schizophrenia and depression), which will only add to the difficulties.

One frequent issue, which has been indicated to some degree above, is the extent to which epigenetic therapies will genuinely alter disease progression or even cure a disorder entirely, rather than simply delay symptomatic presentation. For some diseases with an unequivocally grim prognosis such as HD or AD (particularly the early onset form), current therapies are so inadequate that even a few extra years of good-quality healthy life would represent a major improvement. This will affect the risk–benefit equation in favour of repositioning of broad-acting drugs with a side-effect profile that is less than optimal, such as HDAC inhibitors. This is less likely to be true of less catastrophic human disorders, especially where there are existing therapeutic options. A clear example would be a disease such as RA, which – although debilitating – is rarely directly life-threatening, and for which effective but expensive antibody-based therapeutics are available. In such cases, more precisely targeted epigenetic therapies will be required, with strong mechanism-of-action rationales and side-effect profiles that are, at the very least, no worse than those of existing drugs. It seems to us improbable that this can be achieved by targeting promiscuous chromatin modifiers represented by the HDACs and the DNA methyltransferases. Success is more probable through the selective second-generation chromatin targets and requires extensive collaborations in basic research, disease model-
ling, compound development and clinical testing. Whether successful interventions will require monotherapy or combination therapies (as seems the most promising avenue for epigenetic drugs in oncology) also remains to be established. No one sector or organization holds all the skills necessary for success in this endeavour, but effective collaborations between cutting-edge academic laboratories and the commercial sector have the potential to bring major benefits to patients through the development of second-generation epigenetic interventions.

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